MDMA - BINGE USE AND FUNCTIONAL OUTCOMES IN THE RAT

RATCHANEE RODSIRI

BSc. Pharm. (Hons.)

Institute of Neuroscience

School of Biomedical Sciences

University of Nottingham

Thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy

MAY 2009

CONTENTS

		PAGE
Acknowledgement		i
Publications		ii
Abstract		iii
Abbreviations		v
List of figures		vii
List of tables		xi
CHAPTER 1: General introduction		
1.1	**5-Hydroxytryptamine (5-HT; Serotonin)**	
	1.1.1 Synthesis and metabolism of 5-HT	2
	1.1.2 5-HT receptors	3
	1.1.3 5-HT pathways in the CNS	7
1.2	**Dopamine**	
	1.2.1 Synthesis and metabolism of dopamine	9
	1.2.2 Dopamine receptors	11
	1.2.3 Dopaminergic pathways in the CNS	13
1.3	**3,4-Methylenedioxymethamphetamine (MDMA or 'ecstasy')**	16
	1.3.1 The acute effects of MDMA in experimental animals	20
	1.3.2 The acute effects of MDMA in humans	29
	1.3.3 The long-term effects of MDMA in experimental animals	32
	1.3.4 The long-term effects of MDMA in humans	34
	1.3.5 Possible mechanisms of MDMA-induced neurotoxicity	36
1.4	**Learning and memory**	46
1.5	**Involvement of 5-HT in learning and memory**	47
1.6	**Involvement of dopamine in learning and memory**	50
1.7	**Effects of MDMA on learning and memory**	
	1.7.1 Evidence from human studies	52
	1.7.2 Evidence from animal studies	53
1.8	**Novel object discrimination**	55
1.9	**Objectives**	58

CHAPTER 2: The acute effect of single low doses of MDMA on novel object discrimination **PAGE**

2.1 Introduction 61

2.2 Materials and methods 62

2.3 Results 74

2.4 Discussion 80

CHAPTER 3: The acute effects of repeated administration of low doses of MDMA on locomotion, body temperature and extracellular 5-HT in the hippocampus

3.1 Introduction 84

3.2 Materials and methods 86

3.3 Results 101

3.4 Discussion 111

CHAPTER 4: The long-term effects of repeated MDMA administration on memory and 5-HT neurotoxicity

4.1 Experiment 1: Acute effects of repeated MDMA administration to singly housed rats on locomotion and body temperature and the long-term effects on novel object discrimination and neurotoxicity

4.1.1 Introduction 118

4.1.2 Materials and methods 120

4.1.3 Results 124

4.1.4 Discussion 140

4.2 Experiment 2: Acute effects of group housing on the MDMA-induced change in body temperature and the long-term effects on novel object discrimination and neurotoxicity

4.2.1 Introduction 146

4.2.2 Materials and methods 147

4.2.3 Results 149

4.2.4 Discussion 160

CHAPTER 5: Influence of brain tyrosine availability on MDMA-induced 5-HT neurotoxicity **PAGE**

5.1 Introduction 164

5.2 Acute effects of tyrosine depletion on brain tyrosine, dopamine, 5-HT and their metabolite levels

5.2.1 Materials and methods 166

5.2.2 Results 174

5.3 Effects of tyrosine depletion on MDMA-induced 5-HT neurotoxicity

5.3.1 Materials and methods 183

5.3.2 Results 185

5.4 Discussion 193

CHAPTER 6: General discussion

6.1 Introduction 198

6.2 The interpretation of animal data to humans 200

6.3 Does low dose MDMA administration produce long-term 5-HT neurotoxicity? 201

6.4 Mechanisms for memory impairments produced by MDMA 203

6.5 Summary 204

References 206

Appendix I 247

Appendix II 248

Appendix III 249

Appendix IV 250

ACKNOWLEDGEMENTS

I would like to thank my supervisors Professor Charles Marsden and Professor Kevin Fone for their kindly support throughout my PhD; without their help and patience much of what I have achieved would not have been possible. I also wish to thank Professor Richard Green for his knowledge of MDMA and his kindly guidance during my study. Thanks to the School of Biomedical Sciences for allowing me to use the department facilities.

I also wish to thank many people within School of Biomedical Sciences, Karen Swift for her HPLC expertise, Clare Spicer for her help with microdialysis technique, Professor Fran Ebling for radiotelemetry technique, Ian Topham for helping with all behavioural tests and all the staff who have been a great help during my study.

Special thanks to my family especially my parents for their understanding and support and my sister who inspired me to always do my best. Thanks to all academic staff in The Faculty of Pharmaceutical Sciences, Chulalongkorn University especially Associate Professor Boonyong and Associate Professor Mayuri Tantisira for their kindly advice and support before and during my PhD.

Finally thanks to the Royal Thai Government under the program "Strategic Scholarships for Frontier Research Network" for funding and opportunity.

PUBLICATIONS

Abstract

Rodsiri R., Fone K.C.F., Green A.R., Marsden C.A. (2007) Tyrosine depletion fails to alter MDMA-induced loss of serotonin in the rat brain. *Journal of Psychopharmacology*, 21(7) (Supplement), MG09, A31.

Rodsiri R., Marsden C.A., Fone K.C.F., Green A.R. (2008) Changes in activity and temperature fail to correlate with 5-HT release following repeated MDMA administration in rats. *Journal of Psychopharmacology*, 22(5) (Supplement), A65, A81.

Rodsiri R., Marsden C.A., Fone K.C.F., Green A.R. (2008) Changes in activity and temperature fail to correlate with 5-HT release following repeated MDMA administration in rats. *Fundamental and Clinical Pharmacology*, 22 (Supplement 2), SCP028, P 124.

Papers

Rodsiri R., Green A.R., Marsden C.A., Fone K.C.F. (2009) Effect of acute brain tyrosine depletion on MDMA-induced changes in brain 5-HT (Journal of Psychopharmacology accepted with minor correction)

ABSTRACT

3,4-Methylenedioxymethamphetamine (MDMA) use has increased dramatically and more intensive patterns of use such as binging have become common. This thesis pays particular attention to the translation of animal data to humans by examining low doses and binge type repeated regimen of MDMA in the rat. The functional effects especially acute and long-term effects on memory have been investigated together with the measurement of changes in 5-HT and dopamine to investigate the possible link of these neurotransmitters and the functional effects of MDMA.

The acute effect of single low doses of MDMA on memory was initially examined and it was shown that MDMA (3 mg/kg) acutely disrupted novel object discrimination when given 30 min before the test. However there was no change in 5-HT and dopamine in the hippocampus, striatum and frontal cortex 150 min after MDMA administration. The combined techniques of radiotelemetry and *in vivo* microdialysis were used to examine effects of 'binge-type' repeated low dose MDMA administration (3 or 6 mg/kg i.p. x 3 every 2 h). Locomotor activity, body temperature and 5-HT release in the hippocampus were simultaneously measured in the same animal during MDMA administration. MDMA (3 x 6 mg/kg) increased locomotor activity after each injection. In addition MDMA (3 x 3 mg/kg) produced hypothermia following each injection while MDMA (3 x 6 mg/kg) changed thermoregulation as it decreased body temperature after the first injection and then increased body temperature after the second to a maximum of +1.3 °C after the third injection. Both 'binge' doses of MDMA however increased extracellular 5-HT in the hippocampus after each injection and there was no correlation between 5-HT release in the hippocampus and changes either in locomotor activity or body temperature.

The long-term effect of repeated administration of low doses of MDMA (3 or 6 mg/kg i.p. x 3 every 2 h) on memory was investigated using novel object discrimination 2 weeks after treatment. To imitate the single housing condition

used in radiotelemetry experiments, rats were individually housed during drug treatment. MDMA (3 x 6 mg/kg) caused impairment of novel object discrimination but there was no change in 5-HT, dopamine and their metabolites in the hippocampus, striatum and frontal cortex 2 weeks after MDMA treatment suggesting no contribution of either 5-HT or dopamine loss to the MDMA-induced memory impairment.

The effects of housing conditions on MDMA-induced changes in body temperature and subsequent 5-HT neurotoxicity were determined. Group housed rats showed a similar pattern of changes in body temperature to singly housed rats measured by radiotelemetry following MDMA (3 x 6 mg/kg) suggesting no effect of the housing condition on MDMA-induced changes in body temperature. MDMA (3 x 6 mg/kg) given to group housed rats however produced loss of hippocampal 5-HT 2 weeks after treatment indicating that MDMA-induced hyperthermia is not an essential factor for MDMA-induced neurotoxicity.

The influence of tyrosine on MDMA-induced 5-HT neurotoxicity was determined by depletion of brain tyrosine availability by giving a tyrosine-free amino acid mixture (1 g/kg twice 1 h apart) to Dark Agouti rats before and after MDMA administration (12.5 mg/kg i.p.). A small increase of tyrosine in the hippocampus and striatum occurred in rats treated with MDMA alone. Although the tyrosine-free amino acid mixture decreased tyrosine in the hippocampus and striatum by more than 50% 2 h after administration, this did not protect against MDMA-induced acute hippocampal and striatal 5-HT depletion and long-term 5-HT loss in the hippocampus indicating no effect of tyrosine on MDMA-induced 5-HT neurotoxicity.

Overall the results of the present study provide extensive evidence for acute and long-term memory impairments following single and 'binge-type' repeated low dose MDMA administration and that these effects may translate effectively to human conditions. The memory impairments appeared to have no link with 5-HT and dopamine thus it is important to focus on other factors involved in the mechanism of MDMA-induced memory impairments.

ABBREVIATIONS

2,3-DHBA	2,3-dihydroxybenzoic acid
5-HIAA	5-hydroxyindole acetic acid
5-HT	5-hydroxytryptamine
5-HTP	5-hydroxytryptophan
8-OH-DPAT	8-hydroxy-2-(di-*n*-propylamino)tetralin
AADC	L-aromatic amino acid decarboxylase
aCSF	artificial cerebrospinal fluid
AUC	area under the curve
C_{max}	maximum plasma drug concentration
CNS	central nervous system
COMT	catechol-*o*-methyl transferase
CSF	cerebrospinal fluid
DOPA	dihydroxyphenylalanine
ECD	electrochemical detection
EDTA	ethylenediamine-tetraacetic acid
GABA	gamma-aminobutyric acid
GSH	glutathione
HHMA	3,4-dihydroxymethamphetamine
HPLC	high performance liquid chromatography
HVA	homovanillic acid
i.p.	intraperitoneal
LTP	long-term potentiation
MAO	monoamine oxidase

MDA	3,4-methylenedioxyamphetamine
MDEA	3,4-methylenedioxy-*N*-ethylamphetamine
MDMA	3,4-methylenedioxymethamphetamine
mRNA	messenger ribonucleic acid
NAC	N-acetylcysteine
NE	norepinephrine
NMDA	N-methyl-D-aspartate
OPA	*o*-phthaldehyde
p.o.	'per os' , orally
PET	positron emission tomography
ROS	reactive oxygen species
SERT	serotonin reuptake transporter
SPECT	single photon emission computed tomography
TPH	tryptophan hydroxylase
VMAT	vesicular monoamine transport protein

LIST OF FIGURES

CHAPTER 1		PAGE
1.1	The metabolic pathway of serotonin	4
1.2	Serotonergic pathways in human and rat brains	8
1.3	The metabolic pathway of dopamine	10
1.4	Dopaminergic systems in human brain	15
1.5	Chemical structures of methamphetamine, MDMA, MDA MDEA and mescaline	19
1.6	Effects of MDMA on the serotonin neuron	22
1.7	Pathways of MDMA metabolism in rats, mice and humans	39
1.8	An integrated hypothesis for the development of selective 5-HT terminal degeneration following MDMA	41
1.9	Overall mechanisms of MDMA-induced 5-HT neurotoxicity	44
1.10	The novel object discrimination paradigm used in this thesis	57

CHAPTER 2		
2.1	Diagram representation of the experimental protocol	65
2.2	Diagram representing the familiarisation and choice trials of novel object discrimination	65
2.3	The oxidation of catecholamine and indoleamine	68
2.4	The components of HPLC-ECD system used in this thesis	69
2.5	An example of the chromatographic separation of dopamine, DOPAC, 5-HT, 5-HIAA and HVA (10^{-7} M each)	70
2.6	Examples of chromatograms from striatum, hippocampus and frontal cortex samples	71
2.7	Linear relation between the concentrations of dopamine, DOPAC, HVA, 5-HT and 5-HIAA and the detector response	72
2.8	Effects of MDMA on the time spent exploring objects during trial 1 and trial 2 of novel object discrimination	76
2.9	The preference index (PI) of time spent exploring the novel versus the familiar objects in the choice trial	77

CHAPTER 3		PAGE
3.1	Diagrammatic representation of the experimental protocol	87
3.2	Combining radiotelemetry and microdialysis to simultaneously record locomotor activity, body temperature and 5-HT release in the hippocampus during repeated MDMA administration	90
3.3	Radiotelemetry data for baseline monitoring of locomotor activity and body temperature	91
3.4	The basic structure of the microdialysis probe	95
3.5	The coordinates of the hippocampus where a microdialysis probe was inserted and the staining of microdialysis probe placement in the rat hippocampus	96
3.6	General arrangement of microdialysis probe perfusion set up	97
3.7	Examples of the chromatographic separation of dopamine, DOPAC, 5-HT, 5-HIAA and HVA (10^{-8} M each) in the mixed standard and 5-HT in the microdialysis samples	99
3.8	Linear relation between the concentration of 5-HT injected onto the column and the detector response	100
3.9	Effect of repeated administration of MDMA on locomotor activity recorded using radiotelemetry	102
3.10	Effect of repeated administration of MDMA on body temperature recorded using radiotelemetry	104
3.11	Maximum changes in body temperature after MDMA	105
3.12	Effect of repeated MDMA administration on extracellular 5-HT in the hippocampus	107
3.13	Examples of correlation between activity and 5-HT, body temperature and 5-HT and activity and body temperature following the third injection	110

CHAPTER 4		PAGE
4.1	Experimental procedure to determine the effects of repeated MDMA administration to singly housed rats	121
4.2	A computer-controlled infra-red activity monitor chamber	123
4.3	Ambulatory and fine activity 1 day before treatment	125
4.4	Ambulatory activity during treatment with either MDMA or saline in the infra-red activity chambers	128
4.5	Fine activity during treatment with either MDMA or saline in the infra-red activity chambers	129
4.6	The effect of repeated MDMA administration on body temperature	131
4.7	Effects of MDMA or saline on ambulatory and fine movements 13 days after treatment	133
4.8	Effects of repeated MDMA administration on the time spent exploring objects during trial 1 and trial 2 of novel object discrimination 2 weeks after treatment in singly housed rats	136
4.9	The preference index (PI) of time spent exploring the novel versus the familiar objects in the choice trial	137
4.10	Experimental procedure to determine the effect of repeated MDMA administration to group housed rats	148
4.11	Ambulatory and fine activity 1 day before treatment	150
4.12	Effect of repeated administration of either MDMA or saline on body temperature	152
4.13	Effects of MDMA or saline on ambulatory and fine movements 13 days after treatment	154
4.14	Effects of repeated MDMA administration on the time spent exploring objects during trial 1 and trial 2 of novel object discrimination 2 weeks after treatment in group housed rats	156
4.15	The preference index (PI) of time exploring the novel versus the familiar objects in the choice trial	157

CHAPTER 5 PAGE

5.1 Diagrammatic representation of experimental protocol for determining acute effects of tyrosine depletion and MDMA 170

5.2 Reaction scheme to illustrate the derivatisation reaction between *o*-phthalaldehyde and primary alkyl amine in the presence of sulfite ions producing *N*-alkyl-1-isoindole sulphonates 171

5.3 An example of the chromatographic separation of tyrosine and norvaline mixed standard and tyrosine in the striatum 172

5.4 Linear relation between the concentrations of tyrosine injected onto the column and the detector response 173

5.5 Effect of the tyrosine-free amino acid mixture given with or without MDMA on tyrosine levels 177

5.6 Effect of acute tyrosine depletion on 5-HT and 5-HIAA levels in the hippocampus 2 h after MDMA administration 179

5.7 Acute effect of tyrosine depletion on 5-HT and 5-HIAA levels in the striatum 2 h after MDMA administration 181

5.8 Experimental procedure for determining the acute effects of tyrosine depletion and/or MDMA on rectal temperature 184

5.9 Effects of the tyrosine-free amino acid mixture given with or without MDMA on rectal temperature 186

5.10 Long-term effect of tyrosine depletion on 5-HT and 5-HIAA levels in the hippocampus 2 weeks after MDMA administration 188

5.11 Long-term effect of tyrosine depletion on dopamine, DOPAC and HVA levels in the hippocampus 2 weeks after MDMA administration 191

LIST OF TABLES

CHAPTER 1		PAGE
1.1	Serotonergic receptor subtypes, distribution in the CNS and behavioural responses	5
1.2	Dopaminergic receptor subtypes, distribution in the CNS and behavioural responses	12
CHAPTER 2		
2.1	Total time spent exploring objects in trial 1 and trial 2	77
2.2	5-HT and 5-HIAA levels in rat hippocampus, frontal cortex and striatum following MDMA administration	79
2.3	Dopamine and its metabolite levels in rat hippocampus, frontal cortex and striatum following MDMA administration	79
CHAPTER 3		
3.1	Correlations between extracellular 5-HT in the hippocampus and either locomotor activity or body temperature and correlation between locomotor activity and body temperature	109
CHAPTER 4		
4.1	Total time spent exploring objects in trial 1 and trial 2 of novel object discrimination in singly housed rats	137
4.2	The long-term effect of repeated MDMA administration on 5-HT and 5-HIAA levels in singly housed rats	139
4.3	The long-term effect of repeated MDMA administration on dopamine and its metabolite levels in singly housed rats	139
4.4	Total time that spent exploring objects in trial 1 and trial 2 of novel object discrimination in group housed rats	157
4.5	The long-term effect of repeated MDMA administration on 5-HT and 5-HIAA levels in group housed rats	159
4.6	The long-term effect of repeated MDMA administration on dopamine and its metabolite levels in group housed rats	159

CHAPTER 5 **PAGE**

5.1 Acute effect of tyrosine depletion on dopamine, DOPAC and HVA levels in the striatum 2 h after treatment 182

5.2 Long-term effect of tyrosine depletion on 5-HT and 5-HIAA levels in the striatum 2 weeks after treatment 190

5.3 Long-term effect of tyrosine depletion on 5-HT and 5-HIAA levels in the frontal cortex 2 weeks after treatment 192

CHAPTER 1

GENERAL INTRODUCTION

3,4-Methylenedioxymethamphetamine (MDMA, ecstasy) is a commonly used drug of abuse. MDMA produces acute 5-HT and dopamine release in experimental animals and causes selective 5-HT neurotoxicity in rats and possibly in human users. There is growing evidence for learning and memory impairments in MDMA users. The translation of effects of MDMA in animal studies to human has been widely debated as the typical doses used to produce neurotoxicity are much greater than those used by humans. The aim of this thesis is to investigate the effects of MDMA on memory with the focus on using doses in the rat that translate to single and 'binge type' repeated low doses of MDMA used by humans. In this chapter 5-HT and dopamine, the neurotransmitters currently most associated with MDMA, are reviewed followed by discussion of MDMA and its acute and long-term pharmacological and functional effects. This includes consideration of the possible association between the effects of MDMA on learning and memory and their link with 5-HT and dopamine. Finally the method used to investigate memory in this thesis, novel object discrimination, is described.

1.1. 5-Hydroxytryptamine (5-HT; Serotonin)

1.1.1 Synthesis and metabolism of 5-HT

5-hydroxytryptamine (5-HT) or serotonin is an indoleamine neurotransmitter which is synthesized from the essential amino acid tryptophan. Tryptophan is competitively transported into the brain via the large neutral amino acid transporter (Fernstrom and Wurtmanm, 1972, Wurtmanm and Melamed, 1981). The synthesis of 5-HT occurs in two steps (Figure 1.1). Firstly, tryptophan is

converted to 5-hydroxytryptophan (5-HTP) by the rate-limiting enzyme tryptophan hydroxylase. The 5-HTP is then decarboxylated by the enzyme L–aromatic amino acid decarboxylase (AADC) to form 5-hydroxytryptamine (5-HT) (Figure 1.1). 5-HT is concentrated in presynaptic storage vesicles by the vesicular monoamine transporter protein (VMAT). Following release into the synaptic cleft, 5-HT may bind to 5-HT receptors or is taken up again by the presynaptic serotonin reuptake transporter (SERT). When returned to the 5-HT terminal, 5-HT is either transferred back into presynaptic storage vesicles or metabolized by the mitochondrial enzyme monoamine oxidase (MAO) and aldehyde dehydrogenase to form 5-hydroxyindole acetic acid (5-HIAA) (Barnes and Sharp, 1999) (Figure 1.1).

1.1.2 5-HT receptors

Fourteen subtypes of 5-HT receptors have been identified, each of which is characterized by a unique structure, pharmacology, pattern of expression and signal transduction pathway (see review Barnes and Sharp, 1999, Green, 2006, Hoyer et al., 1994, 2002). The main subgroups of 5-HT receptors have been named 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7. The 5-HT receptor families are mostly G-protein coupled receptors but 5-HT_3 is a ligand-gated ion channel. The G protein coupled transmembrane receptors can be divided into three types; G-protein coupled to the inhibitory adenylyl cyclase (G_i) (5-$HT_{1A,B,D,E,F}$), G-protein coupled to phosphoinositol hydrolysis (G_q) (5-$HT_{2A,B,C}$) and G-protein coupled to the excitatory adenylyl cyclase (G_s) (5HT_4, 5-HT_6 and 5-HT_7). Electrophysiological studies have demonstrated that 5-$HT_{1A/1B/1D/1E/1F}$ receptor activation produces hyperpolarization whereas 5-

$HT_{2A/2B/2C}$, 5-HT_3, 5-HT_4 and 5-HT_7 receptors elicit depolarization (Hoyer et al 2002). 5-HT receptor subtypes, their distribution in the brain and functions summarised in Table 1.1.

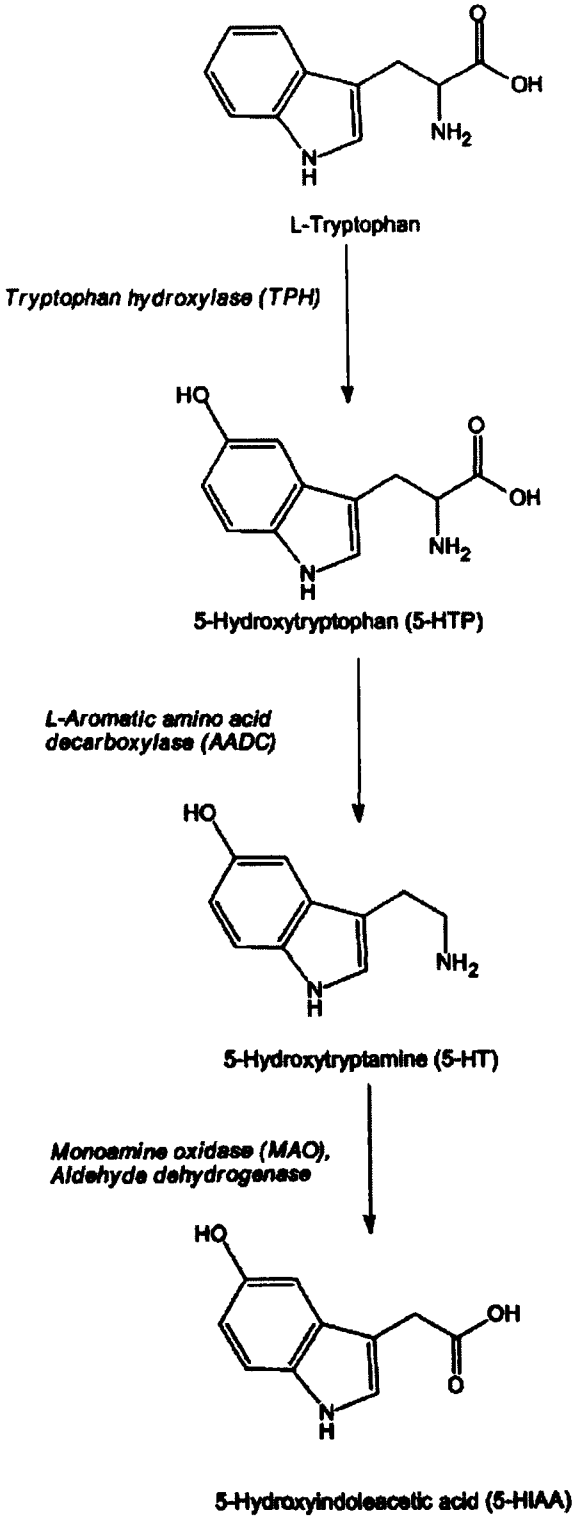


Figure 1.1 The schematic diagram of the metabolic pathway for serotonin

Table 1.1 Serotonergic receptor subtypes, distribution in the central nervous system (CNS) and behavioural responses (Alex and Pehek, 2007, Barnes and Sharp, 1999, Hoyer et al., 1994, 2002, King et al., 2008, Pérez-García et al., 2006).

Subtype	G-protein	Localisation in CNS	Behavioural responses (agonism)
5-HT_1			
5-HT_{1A}	G_i	The raphe nuclei (autoreceptor), limbic system, hippocampus, lateral septum, cortical areas, hypothalamus, neocortex	Serotonin syndrome Hypothermia Hyperphagia Anxiolysis Sexual behaviour (+) Autoinhibition (pre-synaptic) Learning and memory
5-HT_{1B}	G_i	Basal ganglia, substantia nigra (rodent specific)	Locomotor activation Hypophagia Hypothermia (g.pig) Myoclonic jerks (g.pig)
5-HT_{1D}	G_i	Basal ganglia, substantia nigra, nigrostriatal pathway, hippocampus, cortex , dorsal raphe nuclei (autoreceptor) (Guinea pig, pig, calf, monkey, human)	
5-ht_{1E}	G_i		
5-ht_{1F}	G_i	Hippocampus, cortex (cingulate and entorhinal cortices), dorsal raphe	

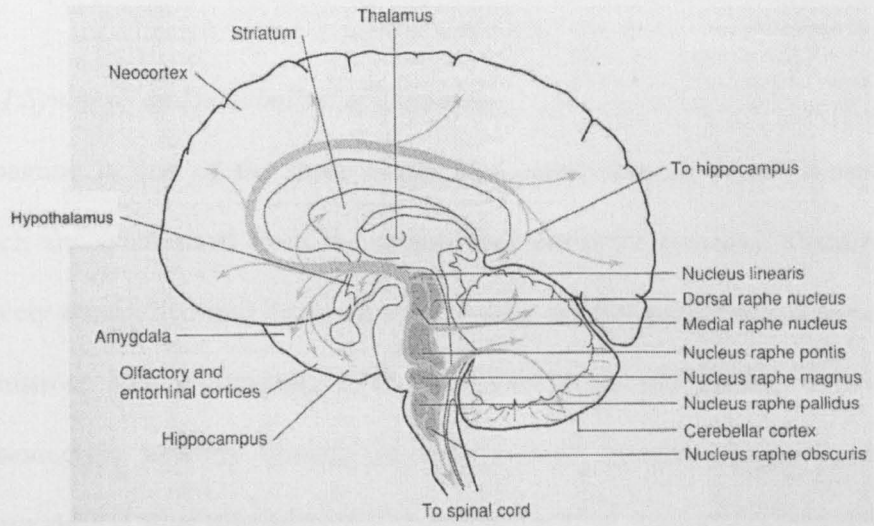
Subtype	G-protein	Localisation in CNS	Behavioural responses (agonism)
5-HT$_2$			
5-HT$_{2A}$	G_q	Cortex, caudate nucleus, nucleus accumbens, olfactory tubercle, hippocampus	Head twitch Wet-dog shake Hyperthermia Discriminative stimulus
5-HT$_{2B}$	G_q	Cerebellum, septum, dorsal hypothalamus, medial amygdala	
5-HT$_{2C}$	G_q	Choroid plexus, cortex, limbic system (nucleus accumbens, hippocampus, amygdale), the basal ganglia (caudate nucleus, substantia nigra)	Hypolocomotion Hypophagia Anxiogenesis Penile erection
5-HT$_3$	Ligand-gated channel	Brain stem nuclei, spinal cord, cortex, hippocampus	Long-term potentiation (LTP) (-) Antagonism : Anti-emetic Anxiolysis Cognition (+) ? Locomotion (-) ? Reward (-) ?
5-HT$_4$	G_s	Substantia nigra, striatum, nucleus accumbens, hippocampus	Anxiolysis Anxiogenesis (antagonist) Cognition (+)
5-HT$_5$			
5-ht$_{5A}$	G_s	Cerebellum, cerebral cortex, hippocampus, hypothalamus, thalamus, pons, striatum, medulla	
5-ht$_{5B}$	unknown		

Subtype	G-protein	Localisation in CNS	Behavioural responses (agonism)
$5\text{-}HT_6$	G_s	Striatum, olfactory tubercle, cerebral cortex, hippocampus, nucleus accumbens	Learning and memory
$5\text{-}HT_7$	G_s	Thalamus, hypothalamus, hippocampus	Circadian rhythms Depression? Learning and memory ?

1.1.3 5-HT pathways in the CNS

The cell bodies of serotonin neurons originate in the raphe nuclei of the brain stem (Figure 1.2). The caudal raphe nuclei, including the raphe magnus, pallidus and raphe obscures project towards the spinal cord and brain stem and form the descending serotonergic systems. Manipulation of the descending serotonergic neurons may affect spinal processing of nociceptive input and motor functions (Berge and Ogren, 1984, Jacobs et al., 2002). The dorsal raphe nucleus, median raphe nucleus and the nucleus pontis are the sources of serotonergic innervations of the forebrain, represent the ascending serotonergic systems (Azmitia and Segal, 1978, Vertes, 1991). The serotonergic neurons originated in the dorsal raphe mainly innervate forebrain structures with a lateral location such as amygdala, striatum, lateral septum, frontal cortex and ventral hippocampus while the projections from median raphe mainly innervate midline structures of forebrain including the dorsal hippocampus and the medial septum (Azmitia and Segal, 1978, Vertes, 1991, 1999) (Figure 1.2).

(A)



(B)

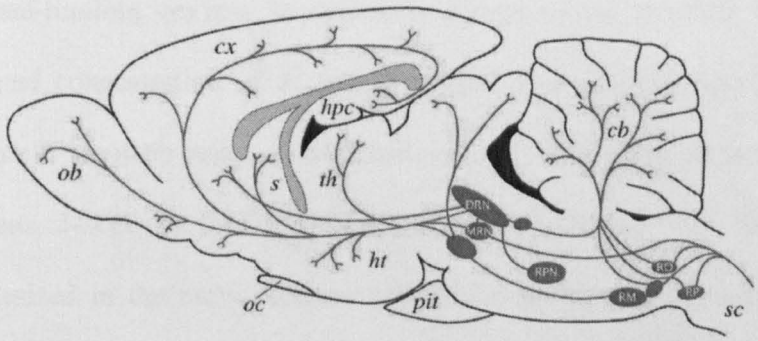


Figure 1.2 Serotonergic pathways in A: human brain (Nestler et al., 2001 p 195) and B: rat brain (Ogren et al., 2008). A: In human brain the cell bodies of serotonergic neurons originate in the brain stem nuclei which are in rostral and caudal clusters. The rostral nuclei, which include the nucleus linearis, dorsal raphe, medial raphe and raphe pontis, form the ascending pathway to the forebrain and the cerebellum. The caudal nuclei, which comprise the raphe magnus, raphe pallidus and raphe obscuris, project the axons to the cerebellum, brain stem and spinal cord. B: The serotonin pathways in rat brain are similar to human. The following abbreviations were used-DRN: dorsal raphe nucleus, MRN: median raphe nucleus, RPN: nucleus raphe pontis, RM: nucleus raphe magnus, RO: nucleus raphe obscurus, RP: nucleus raphe pallidus, cx: cerebral cortex, hpc: hippocampus, ob: olfactory bulb, s: septum, th: thalamus, ht: hypothalamus, oc: optic chiasm, pit: pituitary, cb: cerebellum, sc: spinal cord.

1.2. Dopamine

1.2.1 Synthesis and metabolism of dopamine

Dopamine is one of the three established catecholamine neurotransmitters which are synthesized from the amino acid precursor tyrosine. Tyrosine is actively transported into the brain via the large neutral amino acid transporter (Fernstrom and Wurtmanm, 1972, Grahame-Smith and Parfitt, 1970). In dopaminergic neurons tyrosine is hydroxylated by the enzyme tyrosine hydroxylase to form dihydroxyphenylalanine (DOPA). Tyrosine hydroxylase is the rate-limiting enzyme in dopamine synthesis and as such controls the neuronal concentration of dopamine. DOPA is then decarboxylated by the enzyme L-aromatic amino acid decarboxylase (AADC), the same enzyme that converts 5-HTP to 5-HT, to form dopamine (Figure 1.3). Dopamine is synthesized in the nerve terminal cytoplasm and is kept in storage vesicles following its inward transport by the vesicular monoamine transporter protein (VMAT). Dopamine released into the synaptic cleft may bind to dopamine receptors or be reuptaken into the dopaminergic terminal via the dopamine reuptake transporter. Dopamine is degraded by the mitochondrial monoamine oxidase (MAO) and catechol-*o*-methyl transferase (COMT) to homovanillic acid (HVA) (Figure 1.3).

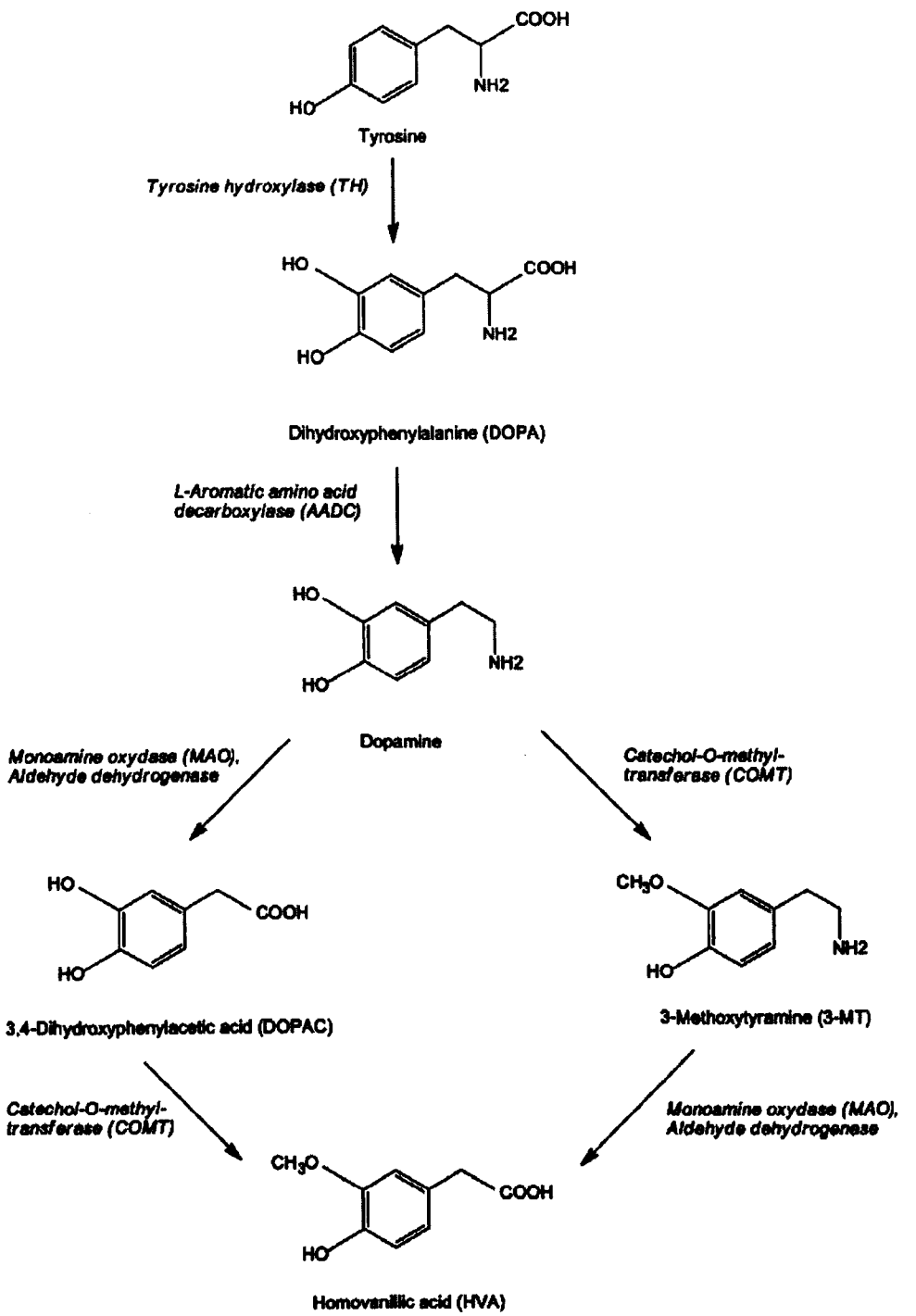


Figure 1.3 The schematic diagram of the metabolic pathway for dopamine.

1.2.2 Dopamine receptors

Brain dopaminergic receptors were originally divided into D_1 and D_2 subtypes on the basis of pharmacological and biochemical evidence (Kebabian and Calne, 1979, Spano et al., 1978). Following the introduction of gene cloning procedures, three further dopamine receptor subtypes were characterized called D_3, D_4 and D_5 (see review Missale et al., 1998, Sokoloff and Schwartz, 1995). The dopaminergic receptors were classified as D_1-like and D_2-like based on the signalling events following G-protein coupling. The D_1-like receptors, which include D_1 and D_5 receptors, couple to G_s and activate adenyl cyclase. The D_2-like receptors, which comprise D_2, D_3 and D_4 receptors are G_i-coupled receptors and inhibit adenyl cyclase (Missale et al., 1998). Dopaminergic receptors in the brain are widely distributed and involved in the control of locomotion, cognition, emotion and affected neuroendocrine secretion. Table 1.2 reviews the dopaminergic receptor subtypes, their distribution in the CNS and functions.

Table 1.2 Dopaminergic receptor subtypes, distribution in the CNS and behavioural responses (Holmes et al., 2004, Missale et al., 1998, Oak et al., 2000).

Subtypes	**G-protein**	**Localisation in the CNS**	**Behavioural responses**
D_1-like	G_s		
D_1		Striatum, amygdala, olfactory tubercle, hippocampus, globus pallidus, hypothalamus, septum, substantia nigra, cerebral cortex	Agonism: Locomotion (+) Rewarding (+) Learning and memory (+)
D_5		Hippocampus, prefrontal cortex, basal ganglia, thalamic and hypothalamic nuclei	D_5R Knockout mice: Learning and memory ? Depression ?
D_2-like	G_i		
D_2		The mesencephalic dopamine cell body regions (autoreceptor), striatum, olfactory tubercle, nucleus accumbens, prefrontal, cingulated, temporal, and enthorhinal cortex, septal, amydala, the granule cells of the hippocampal formation, hypothalamus, substantia nigra pars compacta, ventral tegmental area	Agonism: Locomotion (+) Rewarding (+) Learning and memory (+) Hypothermia Mediate an impairment of prepulse inhibition following amphetamine Pituitary hormone synthesis and secretion (-)

Subtypes	G-protein	Localisation in the CNS	Behavioural responses
D_2-like	G_i		
D_3		Limbic areas, nucleus accumbens, olfactory tubercle, islands of Calleja, substantia nigra pars compacta, ventral tegmentral area, hippocampus, septal	Agonism: Locomotion (-) Anxiolytic effect Rewarding ? Learning and memory (-)
D_4		Prefrontal cortex, amygdala, hippocampus, hypothalamus	D_4R Knockout mice: Hyperactivity Anxiogenic effect?

1.2.3 Dopaminergic pathways in the CNS

There are three dopaminergic systems in the CNS; the nigrostriatal, mesolimbocortical and tuberoinfundibular dopaminergic systems (Figure 1.4). The three dopaminergic nuclei in the brain originate in the substantia nigra pars compacta, ventral tegmental area and the arcuate nucleus. The dopaminergic nuclei of the substantia nigra pars compacta reside in the ventral midbrain and project to the striatum, to form *the nigrostriatal dopamine system.* The striatum, which is a component of the basal ganglia, regulates motor control and the learning of motor programs and habits. Dopamine released by the nigrostriatal system plays a role in the complex circuitry of the basal ganglia and is required for voluntary movement (Figure 1.4).

The dopaminergic nuclei of the ventral tegmental area, found in the ventral midbrain, project to limbic structure including the nucleus accumbens, septum, olfactory tubercle and amygdala and also innervate prefrontal cortex, cingulate and entorhinal cortices and are known as *the mesolimbocortical dopamine systems*. The mesolimbocortical dopamine system plays an essential role in cognition which will be discussed later in *section 1.6.* In addition the dopaminergic connections between the ventral tegmental area and the nucleus accumbens appear to mediate the reinforcing or rewarding properties of drugs of abuse (Figure 1.4).

Finally the dopaminergic nuclei of the hypothalamic arcuate nucleus release dopamine that affects the pituitary gland and constitute *the tuberoinfundibular dopamine system* which controls prolactin release from the anterior pituitary (Figure 1.4).

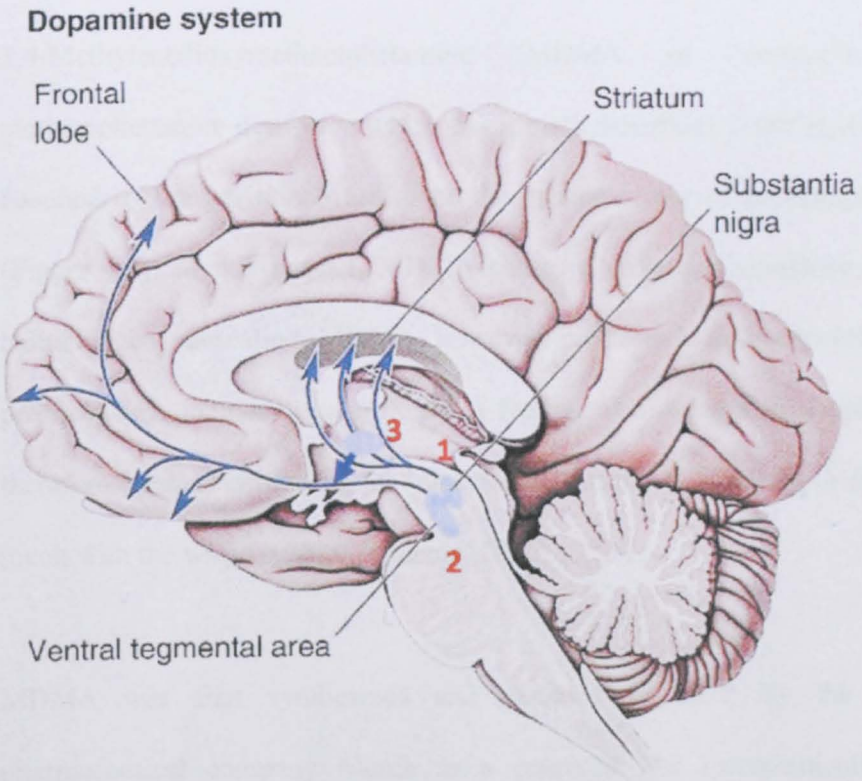


Figure 1.4 Dopaminergic systems in human brain (modified from Bear et al., 2006 p 682). There are three dopaminergic pathways in the brain. Firstly (1) *the nigrostriatal dopamine system*, cell bodies originate in the substantia nigra and project the axons to the striatum. Secondly (2) *the mesolimbocortical dopamine system*, cell bodies originate in the ventral tegmental area and project the axons to limbic structures including the nucleus accumbens, prefrontal cortex and cingulate cortex. Finally (3) *the tuberoinfundibular dopamine system*, dopaminergic cells of the hypothalamic arcuate nucleus release dopamine that affects the pituitary gland.

1.3. 3, 4-Methylenedioxymethamphetamine (MDMA or 'ecstasy')

3,4-Methylenedioxymethamphetamine (MDMA or 'ecstasy') is a methamphetamine derivative which has a methylenedioxy (-O-CH_2-O-) group attached to the position 3 and 4 of the aromatic ring of methamphetamine (Figure 1.5). In this respect, MDMA shares a structural similarity with the hallucinogen, mescaline. MDMA, however, produces a unique psychological profile which includes euphoria and a feeling of closeness to people. It has therefore been classified as an 'entactogen' meaning 'inducing a feeling of touch with the world within' (Nichols, 1986).

MDMA was first synthesized and patented in 1912 by the German pharmaceutical company Merck as a precursor for therapeutically active compounds and was not intended for therapeutic use (Freudenmann et al., 2006). The recreational uses of MDMA firstly emerged in the United States in the mid 1970s. MDMA became popular as a recreational drug in the UK from the 1980s in club dances and parties. In 1977 the UK Home Office listed MDMA as a Class A drug under the Misuse of Drug Act (1971), indicating that it had no medicinal use but serious abuse potential.

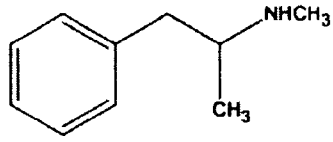
Like other illicit drugs prepared by illegal manufacturers, ecstasy tablets come in different colours, shapes and sizes with doses and purities varying greatly. Several surveys have investigated the composition of ecstasy tablets and reported that most ecstasy tablets contain MDMA and often other ring-substituted amphetamine derivatives such as 3,4-methylenedioxyamphetamine

(MDA) and 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA) (Cole et al., 2002, Parrott, 2004) (Figure 1.5). During the late 1990s, the proportion of MDMA in ecstasy tablets increased to 80-100% (Cole et al., 2002, Parrott, 2004). In 2005, EU surveys revealed that the typical ecstasy tablet contains 30-80 mg of MDMA (EMCDDA, 2007).

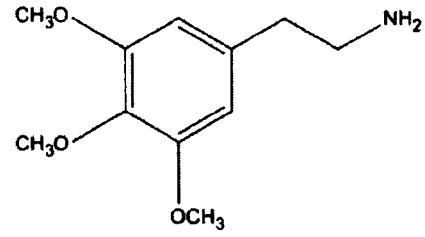
MDMA is usually administered by the oral route in humans and often with multiple dosing in combination with other substances (Hammersley et al., 1999, Parrott et al., 2000, Scholey et al., 2004, Winstock et al., 2001). The prevalence of the illicit use of MDMA has increased over the last decade. According to a UN World drug Report (2008) it is estimated that 9 million people worldwide have taken MDMA (UNDOC, 2008). Approximately 90% of young adults who attend 'raves' and nightclubs reported use of MDMA (Hammersley et al., 1999, Winstock et al., 2001). Although it is claimed that MDMA is safer than other drugs of abuse and that acute fatality caused by MDMA/ecstasy is rare, these do however occur as there were 202 ecstasy-related deaths in England and Wales between 1996 and 2002 according to the National Programme on Substance Abuse Deaths (Schifano et al., 2003).

The first descriptions of MDMA binging in the UK were in a Scottish study from 1993 to 1995 by Hammersley et al (1999). Similar studies of ecstasy use in Australia reported that 35% of subjects (n = 329) had 'binged' on ecstasy in the preceding 6 months (Topp et al., 1999). Winstock et al (2001) reported the levels of typical and binge consumption of ecstasy tablets in the UK which showed an average use of 2.8 tablets per session. A review by Parrott (2005)

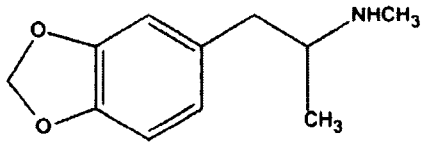
suggested that binge use of MDMA boosts the subjective effects of MDMA possibly by reducing the tolerance effects of repeated usage. Several studies have shown that repeated use of MDMA decreased the desired effects while causing an increase in the side effects of MDMA with more experienced ecstasy users tending to increase the amount of ecstasy taken compared to novice users (Fox et al., 2001, Merill, 1996, Winstock et al., 2001). For example Fox et al (2001) showed that the most experienced ecstasy users (+500 tablets/lifetime) took an average of 3.7 tablets/occasion while the less experienced ecstasy users took 1.8 tablets/occasion. Scholey et al (2004) reported that 100% of novice users took 1-2 tablets/occasion while heavy users generally took either 3-4 tablets (24%) or more than 4 tablets (14%) on each occasion.



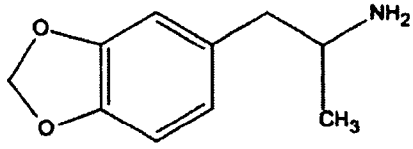
Methamphetamine



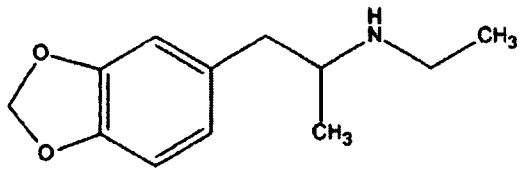
3,4,5-Trimethoxyphenethylamine (mescaline)



3,4-Methylenedioxymethamphetamine (MDMA)



3,4-Methylenedioxyamphetamine (MDA)



3,4-Methylenedioxy-N-ethylamphetamine (MDEA)

Figure 1.5 The chemical structures of methamphetamine, 3,4,5-trimethoxyphenethylamine (mescaline), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxy-N-ethylamphetamine (MDEA).

1.3.1 The acute effects of MDMA in experimental animals

Pharmacological effects

MDMA administration to rats induces an acute and rapid release of 5-HT in rat striatum, medial prefrontal cortex and hippocampus, demonstrated by *in vivo* microdialysis (Gough et al., 1991, Mechan et al., 2002a, Shankaran and Gudelsky, 1999, Yamamoto et al., 1995) followed by a marked decrease of 5-HT levels during the first few hours following MDMA administration (Colado and Green, 1994, Gough et al., 1991, Schmidt, 1987). It was suggested that MDMA-induced 5-HT release is caused by binding of MDMA to the serotonin reuptake transporter (SERT) with consequent transport into the cytoplasm leading to reverse transport of 5-HT into the synapse combined with inhibition of 5-HT reuptake (Berger et al., 1992, Gudelsky and Nash, 1996). Pretreatment with the serotonin reuptake inhibitor, fluoxetine, attenuated MDMA-induced 5-HT release in rat striatum and hippocampus indicating that MDMA-induced 5-HT release involves a carrier-mediated mechanism (Gudelsky and Nash, 1996). In addition MDMA depletes vesicular 5-HT storage by reverse transport of 5-HT from storage vesicles to the cytoplasm via the vesicular monoamine transporter (VMAT) (Partilla et al., 2006). Extracellular 5-HT is further elevated by an inhibition of monoamine oxidase (MAO), the enzyme used to catabolise 5-HT, by MDMA (Leonardi and Azmitia, 1994). Brain 5-HT levels in rats then decrease as MDMA inhibits tryptophan hydroxylase (TPH), the rate-limiting enzyme required for 5-HT synthesis (Johnson et al., 1992, O'Shea et al., 2006, Stone et al., 1987b). Stone et al (1987) demonstrated that TPH activity started to decline in the neostriatum, frontal cortex, hippocampus and

hypothalamus within 15 min of MDMA administration. 5-HT levels however recover within 24 h after MDMA administration (Schmidt 1987). Figure 1.6 summarises the effects of MDMA on 5-HT neurons.

MDMA exposure also causes an acute release of dopamine in the striatum, nucleus accumbens, caudate and hippocampus in rats (Nash and Brodkin, 1991, Nash and Yamamoto, 1992, Yamamoto and Spanos, 1988) and mice (Camarero et al., 2002, Colado et al., 2001). It is possible that the increase in dopamine release results from MDMA-induced 5-HT release which then activates the 5-HT_{2A} receptor to cause an increase in dopamine synthesis and release (Gudelsky and Nash, 1996, Koch and Galloway, 1997, Schmidt et al., 1994).

MDMA has also been demonstrated to stimulate norepinephrine (NE) release from rat brain slices (Fitzgerald and Reid, 1990) and synaptosomal preparations (Rothman et al., 2001). Because of a lack of *in vivo* microdialysis studies measuring NE release, the effects of MDMA on NE are unclear (Green et al., 2003). MDMA also produces acute release of acetylcholine *in vitro* from striatal slices (Fischer et al., 2000). In addition *in vivo* microdialysis demonstrated MDMA-induced acetylcholine release in the prefrontal cortex, striatum and hippocampus (Acquas et al., 2001, Nair and Gudelsky, 2005, Nair and Gudelsky, 2006). Both dopamine D_1 and 5-HT_4 receptors have been shown to mediate MDMA-induced acetylcholine release (Nair and Gudelsky, 2005).

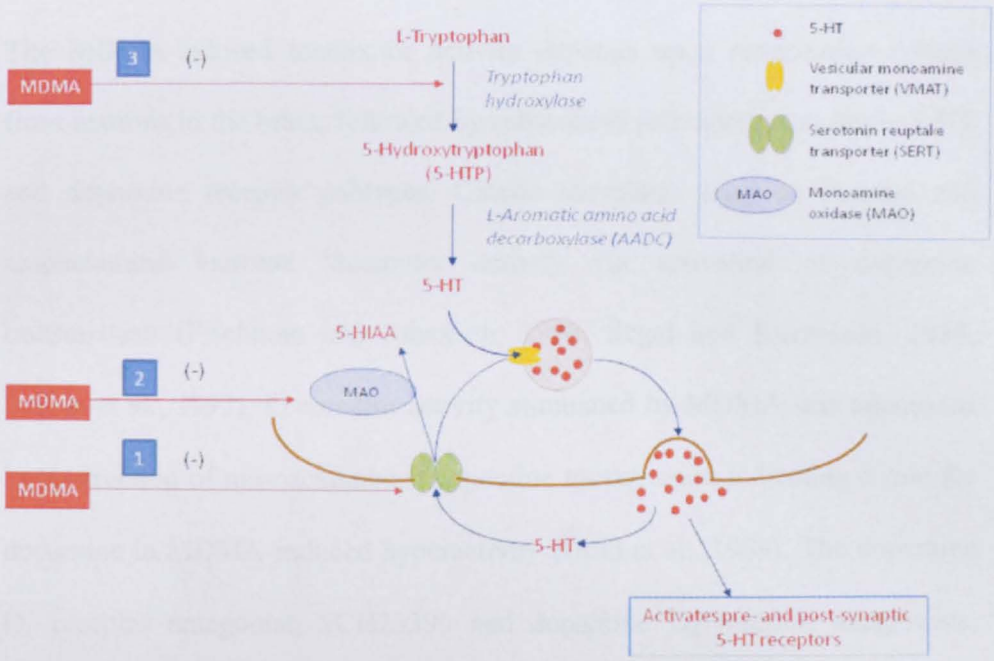


Figure 1.6 Effects of MDMA on a serotonin neuron. (1) MDMA binds to the serotonin reuptake transporter (SERT) causing reverse transport of 5-HT and inhibition of 5-HT reuptake and consequently increases extracellular 5-HT. (2) Extracellular 5-HT is further elevated by inhibition of monoamine oxidase (MAO) by MDMA therefore decreasing 5-HT degradation. (3) MDMA then decreases 5-HT synthesis by inhibiting tryptophan hydroxylase, the rate-limiting enzyme required for 5-HT synthesis, causing a short-term decrease of 5-HT levels.

Effects on locomotor activity and serotonin syndrome

MDMA-induced hyperactivity is a well established acute effect of MDMA. The MDMA-induced locomotor activity depends upon monoamine release from neurons in the brain, followed by subsequent activation of multiple 5-HT and dopamine receptor subtypes. Classic stimulants such as cocaine and amphetamine increase locomotor activity via activation of dopamine transmission (Fischman and Johanson, 1996, Segal and Kuczenski, 1994, Seiden et al., 1993). Locomotor activity stimulated by MDMA was attenuated by destruction of mesoaccumbens dopamine transmission indicating a role for dopamine in MDMA-induced hyperactivity (Gold et al., 1989). The dopamine D_1 receptor antagonist, SCH23390 and dopamine D_2 receptor antagonists, haloperidol and eticlopride attenuate MDMA-induced hyperactivity (Ball et al., 2003). In addition Ball et al (2003) found a positive correlation between MDMA-induced locomotion and activation of striatal neuronal activity. These studies suggest that the locomotor-activating effect of MDMA and other stimulants may share a common underlying mechanism.

Studies by Gold et al (1988) and Spanos and Yamamoto (1989) demonstrated the qualitative difference between the hyperactivity produced by amphetamine and MDMA in rats. Spanos and Yamamoto (1989) reported the symptoms of the serotonin syndrome, a pattern of stereotyped motor behaviour resulting from excessive serotonin release, following MDMA administration to the rats suggesting a role of 5-HT in MDMA-induced hyperactivity. Callaway et al (1990, 1991) showed that preventing MDMA-induced 5-HT release, using fluoxetine pretreatment to block the 5-HT transporter, attenuated the

locomotor-activating effects of MDMA indicating the involvement of MDMA-induced 5-HT release in MDMA-induced hyperactivity. Several studies demonstrated that the 5-$HT_{1B/1D}$ receptor antagonist, GR127935 and 5-HT_{2A} receptor antagonist, M100907 reduced the effect of MDMA on locomotor activity (Bankson and Cunningham, 2002, Fletcher et al., 2002, Herin et al., 2005, Kehne et al., 1996, McCreary et al., 1999) suggesting that activation of 5-HT_{1B} and 5-HT_{2A} receptors by released 5-HT in part at least mediates the effect of MDMA on locomotor activity. In contrast the 5-HT_{2C} receptor antagonist, SB242084 potentiated the activity induced by MDMA (Bankson and Cunningham, 2002, Fletcher et al., 2002).

The mechanisms of MDMA-induced hyperactivity are thus not clearly understood as they involve a complex interaction between the 5-HT and dopamine systems. Ball and Rebec (2005) suggested that the blockade of 5-HT_{2A} receptors reduced MDMA-induced locomotor activity by altering 5-HT_{2A} regulation of dopamine transmission in the striatum as previous studies had shown that 5-HT_{2A} receptors located on dopamine neurons, modulate basal as well as stimulant-induced, dopamine neurotransmission (Gobert et al., 2000, Lucas and Spampinato, 2000). Conversely Fletcher et al (2006) suggested that 5-HT_{2C} antagonism facilitated MDMA-induced dopamine release within the ventral tegmental area.

Effects on body temperature

MDMA-induced changes in body temperature are markedly influenced by ambient temperature. Rats housed in normal experimental conditions (20-22 °C) and high ambient temperature show hyperthermia when given MDMA while low ambient temperature (10, 11 °C) in association MDMA causes hypothermia (Broening et al., 1995, Dafters, 1994).

The mechanisms of MDMA-induced hyperthermia are unclear however both 5-HT and dopamine are likely to be involved. It was demonstrated that the 5-HT_2 receptor antagonists, ketanserin and MDL11939, and the 5-HT_{2A} receptor antagonist, M100907, prevent hyperthermia following MDMA indicating the involvement of 5-HT in MDMA-induced hyperthermia (Herin et al., 2005, Schmidt et al., 1990). However the literature is inconsistent regarding the role of 5-HT in hyperthermia evoked by MDMA as several studies found that pretreatment with fluoxetine, which inhibits MDMA-induced 5-HT release, had no effect on the hyperthermic response to MDMA (Berger et al., 1992, Malberg et al., 1996, Mechan et al., 2002a, Schmidt et al., 1990). Moreover several compounds that block 5-HT receptors, including the 5-$HT_{1/2}$ receptor antagonist, methysegide, 5-HT_{2A} receptor antagonist, MDL100907 and 5-HT_{2C} receptor antagonist, SB242084, failed to prevent the MDMA-induced hyperthermia (Mechan et al., 2002a). The dopamine D_1 receptor has been suggested to be important in MDMA-induced hyperthermia as Benamar et al (2008) and Mechan et al (2002a) showed that the dopamine D_1 receptor antagonist, SCH23390, effectively prevented the hyperthermic response of MDMA while the dopamine D_2 receptor antagonist, remoxipride, had no effect

(Mechan et al., 2002a). Recently a study by Shioda et al (2008) demonstrated that the 5-HT_{2A} receptor antagonists, ritanserin, ketanserin and R-96544 protected against MDMA-induced hyperthermia while the 5-HT_{1A} receptor antagonist, WAY-100635, 5-$HT_{2B/2C}$ receptor antagonist, SB206553 and 5-HT_{2C} receptor antagonist, SB242084 did not prevent this effect of MDMA. In addition the dopamine D_1 and D_2 receptor antagonist, haloperidol, blocks MDMA-induced hyperthermia, but the dopamine D_2 receptor antagonists, sulpiride and L-741626 showed no effect (Shioda et al., 2008). In agreement with Benamar et al (2008) and Mechan et al (2002a), Shioda et al (2008) also showed that the dopamine D_1 receptor antagonist, SCH23390, can prevent MDMA-induced hyperthermia. Taken together, it is likely that MDMA-induced hyperthermia is mediated by 5-HT_{2A} and dopamine D_1 receptors.

Many studies showed a close correlation between MDMA-induced hyperthermia and subsequent neurotoxicity (Baumann et al., 2008a, Green et al., 2004, Malberg and Seiden, 1998, Sanchez et al., 2004). Schmidt et al (1990) and Broening et al (1995) showed that preventing hyperthermia by decreasing ambient temperature can protect against MDMA-induced neurotoxicity. In addition there were several compounds that prevent or attenuate MDMA-induced hyperthermia such as α-methyl-*p*-tyrosine, the dopamine synthesis inhibitor, haloperidol, the dopamine antagonist, and MK-801 (dizocilpine), the *N*-methyl-D-aspartate (NMDA) glutamate receptor antagonist, which can also protect against MDMA-induced 5-HT neurotoxicity (Colado et al., 1993, 1999b, Farfel and Seiden, 1995, Hewitt and Green, 1994, Malberg et al., 1996).

Colado et al (1999a) suggested that MDMA-induced hyperthermia contributes to subsequent 5-HT neurotoxicity through the enhancement of free radical formation in the brain as giving clomethiazole, a gamma-aminobutyric acid A ($GABA_A$) agonist, with MDMA prevented both an acute MDMA-induced hyperthermia and the increase of free radical formation although clomethiazole has no free radical scavenging effect on its own. However if the hyperthermia of the rats was maintained following treatment with MDMA clomethiazole failed to decrease free radical formation suggesting that an increase of body temperature plays a role in the production of free radicals in the brain of MDMA-treated rat (Colado et al., 1999a).

Although there were several studies showing a relationship between MDMA-induced hyperthermia and subsequent 5-HT neurotoxicity, some studies revealed that the compounds which protected against the neurotoxicity of MDMA did not modify body temperature, examples are α-phenyl-*N*-tertbutyl nitrone, a free radical trapping nitrone and fluoxetine (Colado et al., 1997, Sanchez et al., 2001). Moreover while keeping the animals normothermic following MDMA, α-methyl-*p*-tyrosine then failed to protect against MDMA-induced neurotoxicity (Yuan et al., 2002). Taken together, MDMA-induced hyperthermia is one of several factors that promote MDMA-induced neurotoxicity.

Effect on cardiovascular systems

MDMA also produces effects on the cardiovascular systems as MDMA has been shown to produce tachycardia, arrhythmia and vasoconstriction (Fitzgerald and Reid, 1994, Gordon et al., 1991). Recently Badon et al (2002) reported that repeated MDMA administration altered cardiovascular function in rats. Effects of MDMA on cardiovascular systems have been suggested to be mediated by effects of MDMA on peripheral noradrenergic systems as MDMA can displace noradrenaline from adrenergic nerve terminals, competitively block the noradrenaline transporter and directly bind to peripheral and central α_2-adrenoceptors (Al-Sahli et al., 2001, Fitzgerald and Reid, 1993, Lavelle et al., 1999, McDaid and Docherty, 2001).

Anxiety and memory

There are a few studies reporting an acute effect of MDMA administration on anxiety-related behaviours. Morley and McGregor (2000) demonstrated that MDMA (1.25-5 mg/kg) produced an anxiogenic effect in the elevated plus-maze while MDMA (5 mg/kg) had an anxiolytic effect on social interaction. Similarly Ho et al (2004) showed that a single dose of MDMA (7.5 mg/kg) caused an anxiogenic profile in the elevated plus-maze 30 min after treatment while the higher dose of MDMA (15 mg/kg) produced an anxiolytic effect. In agreement with studies in mice the lower dose of MDMA produced an anxiogenic effect while the higher dose of MDMA reduced anxiety in the elevated plus-maze test (Lin et al., 1999, Navarro and Maldonado, 2002). Recently Kindlundh-Hogberg et al (2007) showed that binge administration of MDMA (3 x 5 mg/kg) increased the activity in the centre of the open-field

arena in rats indicating a decrease of anxiety following MDMA. In addition to the anxiety-related behaviours produced by MDMA, several studies showed acute memory impairments in various cognitive models following MDMA administration in rats and rhesus monkeys (Braida et al., 2002, Frederick and Paule, 1997, Harper et al., 2005, Marston et al., 1999, Taffe et al., 2001). The effects of MDMA on memory will be reviewed in *section 1.7.*

1.3.2 The acute effects of MDMA in humans

In humans, serum levels of MDMA peak 2 h after administration, although there are detectable levels after 15 min (de la Torre et al., 2000, Helmlin et al., 1996, Mas et al., 1999). Pharmacokinetic studies in humans have shown that repeated administration of MDMA presents non-linear pharmacokinetics (de la Torre et al., 2000). This is because MDMA is a CYP 2D6 enzyme inhibitor which causes an inhibition of its own metabolism (Delaforge et al., 1999) and consequently increases the area under the plasma concentration-time curve (AUC) and maximum plasma drug concentrations (C_{max}) after a second dose of MDMA following a 24 h interval (Farre et al., 2004).

Recreational ecstasy users have reported acute psychological effects of MDMA including euphoria, sharpened sensory perception, greater sociability, extraversion, heightened sense of closeness to other people, greater tolerance of views and feelings, increase in wakefulness, and postponement of fatigue and sleepiness (Cohen, 1995, Davison and Parrott, 1997, McCann et al., 1996). However there were subacute adverse effects of MDMA ingestion reported including depression, irritability, panic attacks, visual hallucinations and

paranoid delusion (Brown and Osterloh, 1987, Creighton et al., 1991, Davison and Parrott, 1997). MDMA users reported feeling significantly more depressed, unpleasant, sad, abnormal and unsociable than non user controls 7 days after use (Parrott, 2002, Parrott and Lasky, 1998). Topp et al (1999) reported various problems during the post-ecstasy recovery period including energy loss, irritability, muscle aches and trouble sleeping. Curran (2000) also reported midweek low mood in 83% of 469 MDMA users using a questionnaire survey. These mood and other psychobiological problems in the days following MDMA are probably due to the acute monoaminergic depletion already described.

MDMA users have also reported mild signs of the serotonin syndrome including hyperactivity, mental confusion, hyperthermia and jaw clenching (Davison and Parrott, 1997, Parrott and Lasky, 1998). The serotonin syndrome is caused by drug induced excess of intrasynaptic 5-HT. The symptoms include agitation, hyperreflexia, fever, tachycardia, shivering, clonus, myoclonus, ocular oscillations and tremor plus symptoms mentioned above (Gillman, 1999, Huether et al., 1997). The presence of the serotonin syndrome in an MDMA user is indicative of an acute increase in 5-HT release following MDMA ingestion.

The acute adverse physiological effects of MDMA that occur during the peak period after MDMA ingestion in humans include an increase in tension manifested as muscular tension, jaw clenching, tooth grinding and constant restless movement of the legs (McCann et al., 1996, Peroutka, 1987, Shulgin,

1986). Moreover, MDMA also affects the sympathetic nervous system resulting in an acute elevation of heart rate and blood pressure (Vollenweider et al., 1998).

Acute MDMA administration also produces hyperthermia in humans. MDMA ingestion has been reported to increase body temperature to 43 °C (Connolly and O'Callaghan, 1999, Irvine et al., 2006, Mallick and Bodenham, 1997). Davison and Parrott (1997) showed that approximately 85-90% of recreationally MDMA users reported an increase in body temperature and increased sweating and dehydration. Recently Irvine et al (2006) reported a moderate increase of body temperature as well as blood pressure and heart rate in MDMA users at a dance party with the increase of body temperature associated with the highest MDMA plasma concentrations. MDMA-related hyperthermia should be a concern as it can lead to other fatal toxicological problems including acute renal, hepatic, or cardiac failure, rhabdomyolysis and disseminated intravascular coagulation (Cohen, 1996). Similarly to the mechanism in rats, the mechanism involved in MDMA-induced hyperthermia in humans is likely to involve the 5-HT_{2A} receptor as pretreatment with the 5-HT_{2A} receptor antagonist, ketanserin (50 mg p.o.), attenuates the MDMA-induced increase in body temperature (Liechti et al., 2000).

1.3.3 The long-term effects of MDMA in experimental animals

Long-term effects on 5-HT and other neurotransmitter systems

MDMA has been suggested to be a selective neurotoxin of serotonergic neurons in rats (Gibb et al., 1990), guinea pigs (Battaglia et al., 1988, Saadat et al., 2004) and non-human primates (Ricaurte et al., 1988) while MDMA is a selective neurotoxin of dopaminergic neurons in mice (Logan et al., 1988, O'Callaghan and Miller, 1994, O'Shea et al., 2001). Single and multiple MDMA administration produce long-term depletion of 5-HT and 5-HIAA in rats (For example Baumann et al., 2008a, Commins et al., 1987, Ludwig et al., 2008, Slikker et al., 1988). Studies using [^{3}H]paroxetine binding to measure the presynaptic serotonin reuptake transporter (SERT) showed that MDMA administration decreased SERT binding indicating a decrease in the number of serotonergic axon terminals (Battaglia et al., 1987, Broening et al., 1995, Hewitt and Green, 1994, O'Shea et al., 1998). In addition, immunochemical analysis showed a marked loss of fine serotonergic axons in the rat neocortex, striatum and hippocampus following MDMA administration while cell bodies and thick-beaded axonal fibers were not damaged suggesting the subsequent regeneration of the serotonergic neurons (O'Hearn et al., 1988). The rate of neuronal recovery following neurotoxic MDMA administration was shown to be region-dependent. For example Scanzello et al (1993) showed that the 5-HT content in the hypothalamus of the rat first recovered within 8 weeks while hippocampal and striatal 5-HT levels returned to control levels within 32 weeks. Generally in rats, full recovery can be shown in most studies and most brain regions within 1 year (Battaglia et al., 1988, Sabol et al., 1996) while in

non human primates MDMA-induced neurotoxicity is more sensitive and more persistent as neurotoxicity in most neocortical regions and the hippocampus persisted for 7 years after treatment (Fischer et al., 1995, Hatzidimitriou et al., 1999, Ricaurte et al., 1992). The possible mechanisms of neurotoxicity are reviewed later in *section 1.3.5.*

It is well established that MDMA produces a specific neurotoxicity to 5-HT neurons in the rats but there are no long-term changes in dopamine levels or the number of dopamine transporters following MDMA administration (Logan et al., 1988, Kindlundh-Hogberg et al., 2007). In contrast MDMA has a selective dopaminergic neurotoxicity with no long-term effect on 5-HT in mice (Logan et al., 1988, O'Callaghan and Miller, 1994, O'Shea et al., 2001, Kindlundh-Hogberg et al., 2007). A neurotoxic regimen of MDMA also has no long-term effects on tissue norepinephrine levels in either rat or monkey (Battaglia et al., 1987, Insel et al., 1989, Slikker et al., 1988) and no change in the density of catecholamine uptake sites labeled by [^{3}H]mazindol (Battaglia et al., 1987, 1991).

Long-term behavioural effects

Morley et al (2001) showed anxiety-like behaviour in emergence, elevated plus maze and social interaction tests 3 months after MDMA administration to rats. In addition Fone et al (2002) found an increase in the anxiety response in open field behaviour and social interaction 29 days after MDMA administration. In contrast Mechan et al (2002b) reported an anxiolytic response in open field behaviour and the elevated plus maze 73 to 80 days after a neurotoxic dose of

MDMA in rats. There have been several studies reporting long-term effects of MDMA on learning and memory in various cognitive models (Broening et al., 2001, Piper and Meyer, 2004, Sprague et al., 2003, Williams et al., 2003) which will be reviewed in *section 1.7*

1.3.4 The long-term effects of MDMA in humans

Long-term effects on 5-HT and other neurotransmitter systems

Studies of serotonergic neurotransmission in humans have relied upon indirect methods such as the measurement of serotonin metabolites in cerebrospinal fluid and the use of neuroendocrine challenge tests such as d-fenfluramine and L-tryptophan (Gerra et al., 1998, McCann et al., 1999a). Significantly lower levels of the cerebrospinal 5-HIAA have been found in abstinent MDMA users however there was no difference in the prolactin response to L-tryptophan challenge (McCann et al., 1994). Verkes et al (2001) found a significant reduction in the cortisol response to fenfluramine in both moderate and heavy MDMA users. In addition a study from Gerra et al (2000) demonstrated that the cortisol response recovered after 12 months abstinence from MDMA while the prolactin response remained significantly reduced.

Neuroimaging techniques have been applied to study the serotonergic system in the brain of humans with a history of MDMA use. These studies have focused on markers of serotonergic integrity including the 5-HT reuptake transporter as a potential marker for axons and terminals and the 5-HT_{2A} receptor as a potential indirect marker reflecting changes in synaptic 5-HT

release. Findings from positron emission tomography (PET) and single photon emission computed tomography (SPECT) have consistently revealed reductions in 5-HT reuptake transporter ligand binding in heavy and recently abstinent MDMA users (Buchert et al., 2004, McCann et al., 1998, 2005, Reneman et al., 2001a, Semple et al., 1999). Studies using [^{123}I]R91150 SPECT, a specific, reversible ligand of the 5-HT_{2A} receptor, showed that 5-HT_{2A} receptor binding was reduced in recent MDMA users reflecting acute receptor down-regulation by ongoing 5-HT release while it increased in abstinent users for occipital regions (Reneman et al., 2002).

Consistent to studies in rats, MDMA produced specific 5-HT neurotoxicity without depleting dopamine in humans as homovanillic acid (HVA), a dopamine metabolite in cerebrospinal fluid (CSF), was found in the normal range in regular MDMA users (McCann et al., 1994, 1999a). In addition Semple et al. (1999) demonstrated normal dopamine transporter binding in the human lenticular nuclei, studied by SPECT with [^{123}I]β-CIT, in regular MDMA users whereas cortical 5-HT transporter binding was decreased. Consistent with studies in rats, evidence in humans indicates that MDMA does not produce dopaminergic neurotoxicity.

Long-term behavioural effects

Long-term psychological effects following recreational use of MDMA in humans are well documented and are reported to persist long after cessation of drug use (Bolla et al., 1998, McCann and Ricaurte, 1991, 1992, McCann et al., 1996, 1999b, McGuire, 2000). The serious chronic effects include psychiatric

disorders such as anxiety, depression, paranoid psychosis and panic disorder (Benazzi and Mazzoli, 1991, Curran and Travill, 1997, Henry, 1996, McCann et al., 1996, Schifano, 1991). In addition there was a higher incidence of anxiety-related disorders among MDMA users (McCann and Ricaurte, 1991, Morgan, 2000, Parrott, 2001). Several studies of MDMA use in humans showed impairments in memory and learning and this will be discussed in *section 1.7.*

1.3.5 Possible mechanisms of MDMA-induced neurotoxicity

To date, although MDMA causes selective serotonergic neurotoxicity in rats and possibly humans, the mechanisms of this neurotoxicity remain unclear and there are many hypotheses that attempt to explain how the toxicity occurs. These include MDMA-induced free radical formation, toxic metabolites of MDMA and the involvement of dopamine and tyrosine.

MDMA-induced free radical formation

There is a sizable amount of evidence indicating that MDMA-induced neurotoxicity is associated with the ability of MDMA to promote the production of reactive oxygen species (ROS). It has been shown that MDMA administration increases lipid peroxidation, a marker of free radical-induced damage (Colado et al., 1997, Sprague and Nichols, 1995). Additionally, MDMA administration increases the conversion of salicylate to 2,3-dihydroxybenzoic acid (2,3-DHBA), an oxidation product of salicylate (Colado et al., 1997). Moreover, MDMA-induced neurotoxicity can be attenuated by administration of free radical scavenging drugs such as ascorbic

acid, sodium ascorbate, L-cysteine, α-lipoic acid and mazindol (Aguirre et al., 1999, Gudelsky, 1996, Shankaran et al., 1999, 2001). The sources of ROS responsible for toxicity have not been clearly demonstrated.

Metabolites of MDMA

Neurotoxic metabolites of MDMA have been proposed to mediate the neurotoxic effects of MDMA as neither serotonergic nor dopaminergic neurotoxicity were observed after direct injection of MDMA into specific areas of the rat brain (Esteban et al., 2001, Paris and Cunningham, 1992, Schmidt and Taylor, 1988) as well as the C57BL/6J mouse brain (Escobedo et al., 2005) respectively indicating that MDMA may be metabolized outside the brain to neurotoxic metabolites.

The metabolism pathways of MDMA in rats and humans are different. 3,4-methylenedioxyamphetamine (MDA), a major metabolite in rat and mouse, has been suggested to be a neurotoxic metabolite and contribute to MDMA-induced neurotoxicity (Figure 1.7). MDA increased 5-HT release in the nucleus accumbens (Kankaanpaa et al., 1998) and multiple doses of MDA can deplete 5-HT and 5-HIAA and inhibit tryptophan (TPH) activity (Stone et al., 1986, Stone et al., 1987a). In contrast 3,4-dihydroxymethamphetamine (HHMA or N-methyl-α-methyldopamine) is a major metabolite in humans while MDA is minor (De la Torre and Farre, 2004, Pizarro et al., 2004, Segura et al., 2001) (Figure 1.7). Jones et al (2005) have recently synthesized 5-(N-acetylcytein-S-yl)-N-methyl-α-methyldopamine (5-NAC-N-Me-α-MeDA), a likely metabolite

of N-Me-α-MeDA in humans, and demonstrated it to be a potent serotonergic toxicant in rats.

Other potential neurotoxic metabolites are thioethers (glutathione; GSH and N-aceylcysteine; NAC) adduct metabolites of MDMA which also have been shown to be neurotoxic metabolites of MDMA as long-term depletion of 5-HT levels occurred following direct injection of the thioether adducts into the brain of rats (Bai et al., 1999, Miller et al., 1997). Recently Easton et al (2003) have shown that human liver microsomes were able to produce the neurotoxic thioether adducts indicating mechanisms shown in rats can also occur in humans. The GSH adduct of the MDMA metabolite α-methyldopamine, 5-GSH-α-MeDA also decreased cell viability in rat cortical neuronal cultures (Capela et al., 2005). Easton and Marsden (2006) suggested that the neurotoxic effects of MDMA are likely to be a result of a combination of several metabolites (Figure 1.7).

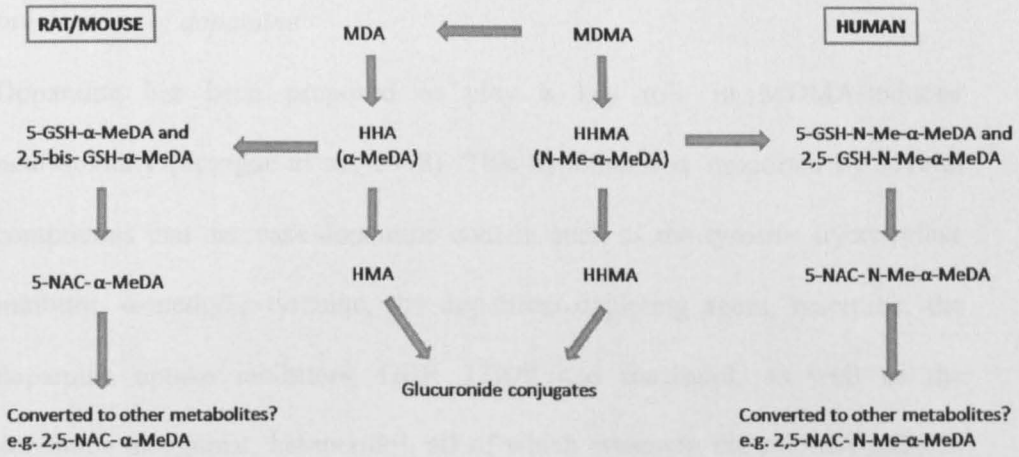


Figure 1.7 Simplified pathways of MDMA metabolism in rats, mice and humans (Easton and Marsden, 2006).

Abbreviations: (1) MDA (3,4-methylenedioxyamphetamine), (2) MDMA (3,4-methylenedioxymethamphetamine, (3) HHA (3,4-dihydroxyamphetamine or alpha-methyldopamine or α-MeDA), (4) HHMA (3,4-dihydroxymethamphetamine or N-methyl-alpha-methyldopamine or N-Me-α-MeDA), (5) HMA (4-hydroxy-3-methoxyamphetamine), (6) HMMA (4-hydroxy-3-methoxymethamphetamine), (7) 5-GSH-α-MeDA (5-[glutathione-S-yl]-alpha-methyldopamine), (8) 2,5-bis-GSH-α-MeDA (2,5-bis-[glutathione-S-yl]-alpha-methyldopamine), (9) 5-GSH-N-Me-α-MeDA (5-[glutathione-S-yl]-N-methyl-alpha-methyldopamine), (10) 2,5-GSH- N-Me-α-MeDA (2,5-[glutathione-S-yl]-N-methyl-alpha-methyldopamine) (11) 5-NAC-α-MeDA (5-[N-acetylcysteine]- alpha-methyldopamine), (12) 5-NAC-N-Me-α-MeDA (5-[N-acetylcysteine]-N-methyl-alpha-methyldopamine), (13) 2,5-NAC-α-MeDA (2,5-[N-acetylcysteine]- alpha-methyldopamine), (14) 2,5-NAC-N-Me-α-MeDA (2,5-[N-acetylcysteine]-N-methyl-alpha-methyldopamine)

Involvement of dopamine

Dopamine has been proposed to play a key role in MDMA-induced neurotoxicity (Sprague et al., 1998). This hypothesis is supported by several compounds that decrease dopamine content such as the tyrosine hydroxylase inhibitor, α-methyl-*p*-tyrosine, the dopamine depleting agent, reserpine, the dopamine uptake inhibitors, GBR 12909 and mazindol, as well as the dopamine antagonist, haloperidol, all of which attenuate the MDMA-induced loss of 5- HT (Hewitt and Green, 1994, Shankaran et al., 1999, Stone et al., 1988). Sprague et al (1998) proposed an integrated hypothesis for the development of selective 5-HT terminal degeneration following MDMA (Figure 1.8). Firstly it has been widely demonstrated that MDMA induces an acute release of both 5-HT and dopamine. The release of dopamine elicited by MDMA involves the activation of $5\text{-HT}_{2A/2C}$ receptors on GABAergic neurones (Cowan et al., 1990, Kita et al., 1990, Nash, 1990, Yamamoto et al., 1995). The increased dopamine is then transported into 5-HT nerve terminals via the serotonin reuptake transporter (SERT) as it has been observed that SERT can also transport dopamine (Faraj et al., 1994, Hrometz et al., 2004, Saldana and Barker, 2004). Dopamine is then metabolized by the mitochondrial enzyme monoamine oxidase (MAO) in the serotonergic terminals to reactive oxygen species (ROS) as the oxidative deamination of monoamine neurotransmitters by MAO produces hydrogen peroxide (H_2O_2) which is subsequently converted to hydroxyl radical (Alves et al., 2007). In addition inhibition of MAO_B using MAO_B inhibitor, L-deprenyl or antisense oligonucleotide targeted at MAO_B decreased MDMA-induced lipid peroxidation and consequently protected against MDMA-induced long-term 5-HT depletion (Falk et al., 2002, Sprague

and Nichols, 1995). Due to the large amount of dopamine transported into 5-HT terminals, MAO_B degradation of this dopamine leads to enhanced production of ROS and further damage to 5-HT terminals.

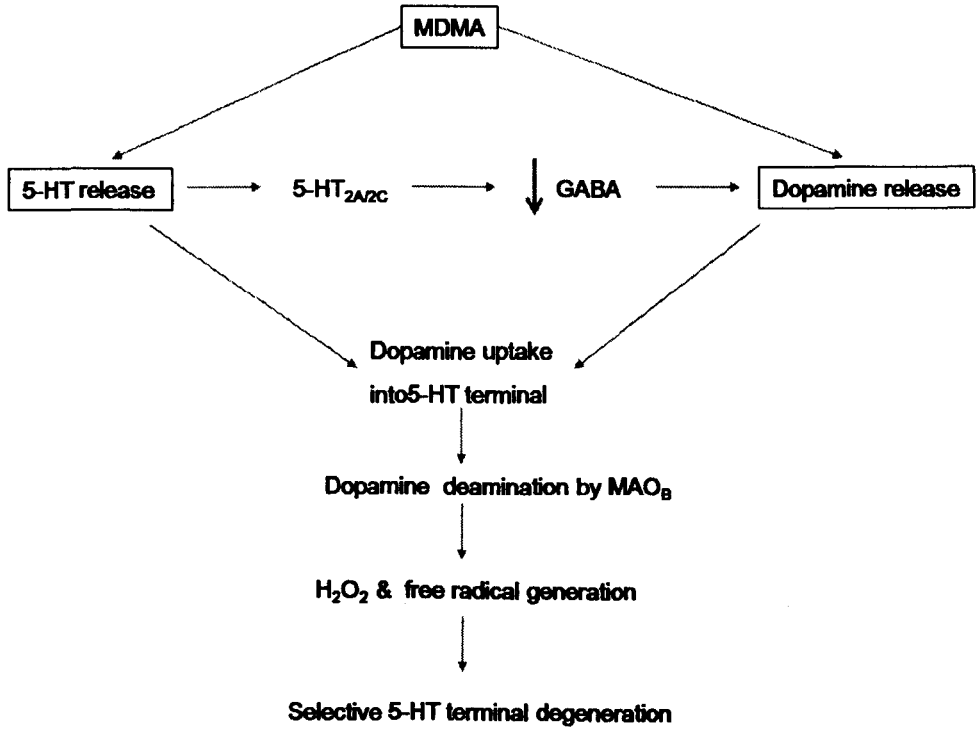


Figure 1.8 An integrated hypothesis for the development of selective 5-HT terminal degeneration following MDMA (Sprague et al 1998).

Involvement of tyrosine

The evidence suggesting involvement of dopamine in MDMA-induced neurotoxicity has been discussed however the role of dopamine on this neurotoxicity in brain areas sparse of dopaminergic innervations, such as the hippocampus, remains unclear. A recent study by Brier et al (2006) suggested the involvement of tyrosine in MDMA-induced 5-HT neurotoxicity as it was demonstrated that MDMA administration markedly increased extracellular tyrosine in the striatum and hippocampus (Breier et al., 2006). Recently Goñi-Allo et al (2008) reported that MDMA administration increased the serum tyrosine concentration suggesting that the rise of tyrosine in the brain may be the consequence of a preceding peripheral tyrosine elevation. Breier et al (2006) suggested that dopamine in brain areas with sparse dopaminergic innervation is produced by a tyrosine hydroxylase-independent mechanism as they demonstrated a conversion of L-tyrosine to DOPA in the presence of hydroxyl radicals using an *in vitro* hydroxyl radical-generating system. Moreover reverse dialysis of α-methyl-*p*-tyrosine, the tyrosine hydroxylase inhibitor, into the hippocampus failed to prevent MDMA-induced 5-HT depletion in the hippocampus indicating this neurotoxicity of MDMA did not involve tyrosine hydroxylase (Breier et al., 2006). The DOPA formed in this way is then converted to dopamine by the aromatic amino acid decarboxylase (AADC) present in 5-HT terminals as the AADC inhibitor, NSD 1015, prevented MDMA-induced dopamine release in the hippocampus and protected against MDMA-induced 5-HT depletion (Breier et al., 2006).

In summary although the precise mechanisms of MDMA-induced 5-HT neurotoxicity are unknown free radical formation is probably a factor. The toxic metabolites of MDMA have been widely demonstrated to be involved in MDMA-induced neurotoxicity and dopamine could be one of the sources of the production of reactive oxygen species (ROS) in 5-HT terminals. It has been suggested that excessive dopamine released by MDMA can then be transported into the 5-HT terminals and metabolized to reactive oxygen species (ROS) by the mitochondrial enzyme monoamine oxidase (MAO). An alternative hypothesis proposes that in brain area sparsely innervated by dopamine tyrosine can be converted to dopamine via a tyrosine hydroxylase-independent mechanism as it was shown that MDMA increased serum and brain tyrosine levels. In addition, as discussed in *section 1.3.1*, MDMA-induced hyperthermia is a factor that influences neurotoxicity as it promotes the production of ROS. The possible mechanisms of MDMA-induced 5-HT neurotoxicity are summarised in Figure 1.9.

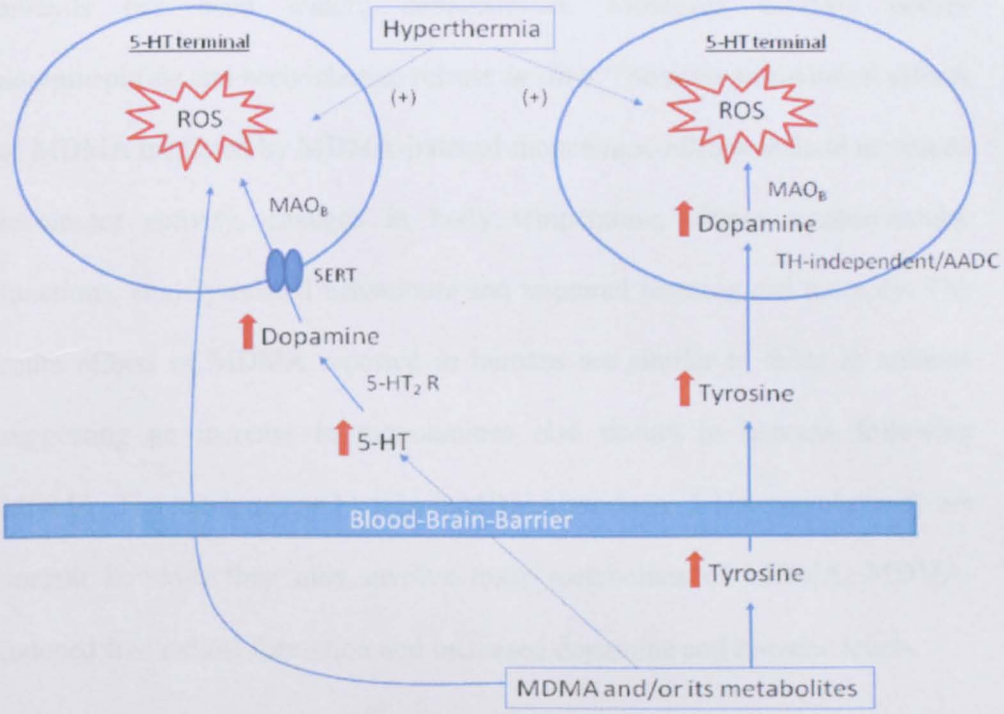


Figure 1.9 An overall mechanism of MDMA-induced 5-HT neurotoxicity. Toxic metabolites produce acute release in 5-HT and dopamine in the brain. Dopamine is then taken up into 5-HT terminals via the serotonin reuptake transporter (SERT) and metabolized by the mitochondrial enzyme monoamine oxidase B (MAO_B) to produces reactive oxygen species (ROS). MDMA also increases plasma and brain tyrosine levels. In the brain sparsely innervated by dopaminergic neurons tyrosine is converted to dopamine via a tyrosine hydroxylase-independent mechanism in the 5-HT terminal. Dopamine is then metabolized by MAO_B and produces ROS. Moreover MDMA and/or its metabolites are oxidized to ROS in the 5-HT terminals.

The evidence for MDMA producing acute 5-HT and dopamine release in animals has been widely demonstrated. Moreover MDMA causes norepinephrine and acetylcholine release *in vitro*. The acute behavioural effects of MDMA mediated by MDMA-induced monoamine release include increased locomotor activity, changes in body temperature, altered cardiovascular functions, anxiety-related behaviours and impaired learning and memory. The acute effects of MDMA reported in humans are similar to those in animals suggesting an increase in monoamines also occurs in humans following MDMA. The mechanisms by which MDMA produces 5-HT neurotoxicity are unclear however they may involve toxic metabolites of MDMA, MDMA-induced free radical formation and increased dopamine and tyrosine levels.

MDMA clearly has its main effects on 5-HT and dopamine systems and 5-HT and dopamine neurons innervate the brain area associated with learning and memory such as the hippocampus and frontal cortex. MDMA therefore may affect learning and memory function by actions in these brain areas. The next section of this chapter reviews the concepts of learning and memory, the importance of 5-HT and dopamine in learning and memory and the effects of MDMA on learning and memory in humans and laboratory animals. The relationship between the effects of MDMA on 5-HT and/or dopamine and impairments of learning and memory in human MDMA users and in laboratory animals following MDMA is also discussed.

1.4. Learning and memory

Learning is the acquisition of alterations in behaviour as a result of particular experience. Memory is the storage of the altered behaviour over time. Memory can be classified, based on the capacity and duration of information storage, as short-term and long-term memory. Short-term memory is the temporary store of information that has limited capacity. Short-term memory can be transformed into a more permanent storage of information which is long-term memory. Long-term memory can be subdivided to declarative and non-declarative (procedural) memory. Declarative memory is the acquisition, retention and retrieval of knowledge that can be consciously recollected including memory for events (episodic memory) and memory for facts (semantic memory). It was proposed that the medial temporal lobe system, including the hippocampus formation (entorhinal cortex, dentate gyrus, areas CA1-CA4 and subiculum), amygdala and parahippocampal cortices, involves a declarative memory system (see review Squire et al., 2004). Non-declarative memory involves the skilled behaviour and the ability to respond appropriately to stimuli through practice. This type of memory is dependent on different brain areas for example memory for skills depends on striatum and cerebellum while emotional learning involves amygdala (see review Milner et al., 1998).

Baddeley and Hitch (1974) introduced the concept of 'working memory' to describe a more dynamic system of short-term memory and defined working memory as the system for the temporary maintenance and manipulation of information, necessary for the performance of such complex cognitive

activities as comprehension, learning, and reasoning (Baddeley, 1996). Working memory has been demonstrated to be dependent on the hippocampus and prefrontal cortex (Laroche et al., 2000). Novel object discrimination task, which is used in this thesis, is one of cognition models for determining working memory as well as recognition memory (Ennaceur and Meliani, 1992). Recognition memory is defined as the ability to discriminate the familiarity of things previously encountered (Aggleton and Brown, 1999). The cognition models used to determine recognition memory include delay matching and nonmatching-to-sample task and novel object discrimination. It is demonstrated that the rhinal and parahippocampal cortices are importance for object-recognition while the contribution of the hippocampus is relatively minor (see review Mumby, 2001).

1.5. Involvement of 5-HT in learning and memory

The ascending serotonergic systems innervate the forebrain including the cerebral cortex, hippocampus, septum and amygdala, all of which represent brain regions associated with various domains of cognition. The involvement of 5-HT in learning and memory has been widely investigated. Depletion of the 5-HT precursor, L-tryptophan, and consequently reduction of brain 5-HT levels impair learning and memory both in humans and rodents (Lieben et al., 2004, Sambeth et al., 2007, Uchida et al., 2007). In addition several 5-HT receptors have been shown to be implicated in the mechanisms of learning and memory (King et al., 2008, Meneses, 2003).

The 5-HT_{1A} receptor has been widely demonstrated to play a role in learning and memory however the mechanisms remain unclear as the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), and 5-HT_{1A} partial agonists, buspirone and tandospirone, produced a range of effects on memory from improvement to impairment in various animal models (see review Meneses and Perez-Garcia, 2007, Ogren et al., 2008). In addition antagonism of 5-HT_{1A} receptors reverses the cognitive impairments induced by 5-HT_{1A} receptor agonists, N-methyl-D-aspatate (NMDA) receptor antagonists and the muscarinic receptor antagonist scopolamine (Forster et al., 1995, Harder and Ridley, 2000, Hirst et al., 2008, Meneses, 2007).

The functions of 5-HT_{1A} receptors are to act as the somatodendritic inhibitory autoreceptor as well as an inhibitory postsynaptic heteroceptor on glutamatergic and cholinergic neurons in the frontal cortex, hippocampus and septum. Therefore stimulation or blockade of pre- or post-synaptic 5-HT_{1A} receptors may produce different results on memory. For example Warburton et al (1997) demonstrated that systemic administration of 8-OH-DPAT, the 5-HT_{1A} receptor agonist, impaired delayed non-matching-to-place (DNMTP) task whereas central infusion of 8-OH-DPAT into the median raphe nucleus improved performance in this task. Considering the post-synaptic 5-HT_{1A} receptors, infusion of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, and partial 5-HT_{1A} receptor agonist, NAN-190, into the rat hippocampus caused an impairment of performance accuracy in DNMTP (Warburton et al., 1997). In addition extra-rhinal cortex infusion of NAN-190 inhibited short-term memory in an inhibitory avoidance task while intra-entorhinal infusion of 8-OH-DPAT

impaired long-term memory (Izquierdo et al., 1998, 1999). Moreover it is suggested that the effects of 5-HT_{1A} receptor antagonists on memory improvement might be mediated by glutamatergic and/or cholinergic systems (Carli et al., 2001, 2006, Luttgen et al., 2005, Madjid et al., 2006).

As the 5-HT_4 receptor is highly expressed in brain regions involved in learning and memory such as basal ganglia and hippocampus and moderately expressed in the frontal cortex and amygdala, this receptor has been suggested to be involved in the mechanisms of learning and memory. It was demonstrated that 5-HT_4 receptor partial agonists, RS67333 and RS17017, improved social and olfactory associative learning, spatial memory and novel object recognition in rats and improved delayed matching-to-sample in macaques (Fontana et al., 1997, Lamirault and Simon, 2001, Marchetti et al., 2004, Terry et al., 1998) while the 5-HT_4 receptor antagonists, SDZ205557 and GR125487, impaired passive avoidance memory in mice (Galeotti et al., 1998). The effects of 5-HT_4 receptor agonists are thought to be mediated by cholinergic neurotransmission as 5-HT_4 receptor agonists reverse scopolamine-induced memory deficit (Galeotti et al., 1998, Micale et al., 2006) and increase acetylcholine efflux in the frontal cortex and hippocampus (Consolo et al., 1994, Matsumoto et al., 2001).

Recently there is a growing evidence for involvement of 5-HT_6 and 5-HT_7 in learning and memory. Woolley et al (2001) demonstrated that antisense oligonucleotides direct against the 5-HT_6 receptor and the 5-HT_6 receptor antagonist, Ro 04-6790, improved performance in Morris water maze.

Moreover several studies demonstrated the roles of 5-HT_6 receptor antagonists in the improvements of memory in novel object recognition and an autoshaping conditioned-response tasks (Hirst et al., 2006, King et al., 2004, Mitchell et al., 2006, Perez-García and Meneses, 2005a, Woolley et al., 2003). In addition the selective 5-HT_7 receptor agonist, AS19, facilitated memory consolidation and modulated mRNA of 5-HT_7 expression in a Pavlovian/instrumental autoshaping task (Perez-García and Meneses, 2005b, Pérez-García et al., 2006).

1.6. Involvement of dopamine in learning and memory

It is well established that *the mesolimbocortical dopamine system* play an essential role in learning and memory (see review Floresco and Magyar, 2006, Missale et al., 1998). Dopamine neurotransmission in the prefrontal cortex has been shown to mediate executive functions such as working memory in primates and rats. Brozowski et al (1979) demonstrated that lesions of dopamine terminals in the prefrontal cortex impaired delayed responding task performance in primates. In addition local administration of the dopamine D_1 receptor antagonists, SCH23390 and SCH39166, into the dorsolateral prefrontal cortex induced a deficit in an oculomotor delay response task in rhesus monkeys while local administration of the dopamine D_2 receptor antagonists, sulpiride and raclopride, had no effect in this task (Sawaguchi and Goldman-Rakic, 1991, 1994). In contrast systemic administration of the dopamine D_2 receptor antagonists impaired working memory in both humans and primates in other studies (Mehta et al., 2004, Von Huben et al., 2006).

In rats lesions to the medial prefrontal cortex, using the selective neurotoxin 6-hydroxydopamine, produced impairment of delayed alternation in T-maze and radial maze performances (Bubser and Schmidt, 1990). In addition Broersen et al (1995) showed that infusion of either the nonselective dopamine antagonist, flupentixol, or the dopamine D_1 receptor antagonist, SCH23390, into the medial prefrontal cortex produced a delayed-independent impairment of delayed matching-to-position in rats. Although blockade of dopamine D_1 receptors in the prefrontal cortex has been shown to impair working memory, several studies have demonstrated that excessive stimulation of the prefrontal cortex dopamine D_1 receptors may also impair performance in a delayed response task in both primates and rats (Bauer and Fuster, 1978, Kesner et al., 1981, Zahrt et al., 1997).

With the extensive evidence for the involvement of serotonergic and dopaminergic systems in learning and memory, short-term and long-term alterations of serotonergic and/or dopaminergic functions caused by MDMA in animals and possibly humans would be predicted to result in learning and memory impairments. The following section reviews the evidence for such impairments in MDMA users and the effects of MDMA administration on learning and memory in the experimental animals. In addition the contribution of changes in 5-HT and/or dopamine produced by MDMA to these impairments is discussed.

1.7. Effects of MDMA on learning and memory

1.7.1 Evidence from human studies

The memory deficit in humans was first described by McCann and Ricaurte (1991) in an individual case study of regular MDMA user who reported poor short- and long-term memory, hallucinations, depression, anxiety and sleep disturbance after ingestion of MDMA for more than 2 years. There is growing evidence for selective impairments of memory associated with chronic and heavy recreational use of MDMA. The most consistent findings of memory deficits in MDMA users are impairments of episodic memory and learning abilities (Gouzoulis-Mayfrank et al., 2000, McCardle et al., 2004, Montgomery et al., 2005, Morgan, 1999, 2002, Parrott and Lasky, 1998, Schifano et al., 1998). Some studies reported deficits in working memory, complex attention, problem solving and test of frontal executive function as well as elevated cognitive impulsivity (Fox et al., 2001, 2002, Gouzoulis-Mayfrank et al., 2000, McCann et al., 1999b, Morgan, 1998, 2002, von Geusau et al., 2004, Wareing et al., 2000, Zakzanis and Young, 2001). Many studies have shown memory impairments related to the reduction of brain 5-HT. For example Bolla et al (1998) found the impairment in verbal and visual memory in MDMA users correlated with the reduction in brain 5-HT as indexed by cerebrospinal 5-HIAA. Renemann et al (2001b) reported an impairment in verbal memory in both recent and ex-MDMA users associated with a decrease of cortical [^{123}I]β-CIT-labelled SERT density in recent but not in ex-MDMA users suggesting that there was a recovery of 5-HT in abstinent users but memory impairments were persistent.

Although the association of MDMA use and memory deficits in humans has been a consistent finding in most studies, the utility of those data is limited by several factors including incomplete history of drug use of the subjects, inappropriate control groups, small group sizes and the difficulty in assessing brain pathology in living human subjects. In addition it is widely reported that MDMA users also consume a variety of other drugs, most notably cannabis, alcohol and cocaine which also caused memory impairments (Rogers and Robbins, 2001, Scholey et al., 2004). It is likely that cognitive impairments reported in MDMA users might be related to other drug use. Studies of the effects of MDMA administration in laboratory animals are important to determine effects of MDMA on learning and memory.

1.7.2 Evidence from animal studies

There is increasing evidence for effects of MDMA on learning and memory in laboratory animals. Marston et al (1999) reported an acute disruption of the delayed non matching-to-place (DNMTP) when MDMA was given twice daily to rats over 3 days in ascending doses (10, 15 and 20 mg/kg). In addition Braida et al (2002) showed an acute effect of single low doses of MDMA (1, 2 or 3 mg/kg) on short-term working memory and impairment of long-term working memory in the eight-arm radial maze task. Harper et al (2005) found that MDMA (2 and 3 mg/kg) impaired the delayed matching-to-sample accuracy when given 10 min before the task. In rhesus monkeys, Frederick and Paule (1997) suggested that MDMA (0.1-1.0 mg/kg) disrupted performance in learning and time estimation more than short-term memory and visual discrimination. However with a higher dose of MDMA (10 mg/kg i.m. twice

daily for 4 days) rhesus monkeys showed impairment of self-ordered spatial search (SOSS) and delayed non-matching-to-sample task (Taffe et al., 2001). To date no study has investigated the neurotransmitters relevant to the acute memory impairments caused by MDMA.

The long-term effects of MDMA on memory in laboratory animals are more controversial and additionally the relationship between impairments in memory and long-term 5-HT depletion caused by MDMA remains unclear. MDMA exposure to very young rats has been reported to cause impairments of sequential and spatial reference memory-based learning using the multiple-T water maze and the Morris water maze while there was little change in 5-HT, dopamine and norepinephrine levels in the hippocampus and frontal cortex (Broening et al., 2001, Williams et al., 2003). On the other hand MDMA administration to adult rats affected spatial learning in the Morris water maze and significantly decreased 5-HT levels in the hippocampus (Sprague et al., 2003). Piper and Meyer (2004) found an impairment of object recognition memory in rats given repeated intermittent MDMA treatment during the periadolescent period, but only a small decrease of 5-HT uptake transporter density in the cortex and hippocampus. In addition, high dose regimen of MDMA also produced a long-lasting disruption of rat memory in the object recognition task (Morley et al., 2001). Other studies have found long-term cognitive impairments caused by MDMA as determined by the delayed non match-to-position task (Marston et al., 1999) and a social memory test (Pompei et al., 2002). Recently Able et al (2006) and Skelton et al (2008) reported an impairment of path integration learning in the Cincinnati water maze in rat

treated with MDMA (4x15 mg/kg) together with long-term brain 5-HT depletion. However, studies in rhesus monkeys suggest that MDMA produces no long-lasting alteration in cognitive performance on several tasks such as SOSS and DMTS even though there is a decrease in 5-HIAA levels in cerebrospinal fluid (CSF) (Frederick et al., 1998, Taffe et al., 2001).

In summary many studies have demonstrated impairments of episodic memory, working memory, executive function as well as learning abilities and attention in recent and abstinent MDMA users. These impairments have also been demonstrated to correlate with a decrease in 5-HT integrity in recent MDMA users however loss of 5-HT was shown to recover in ex-MDMA users. In rats MDMA caused an acute disruption of working memory in various cognitive models. In addition long-term impairments of sequential, spatial and working memory following MDMA administration occurs in rats. The following section reviews the method used to study the effect of MDMA on memory in this thesis, the novel object discrimination task.

1.8. Novel object discrimination

There are various animal models used to investigate effects of MDMA administration on learning and memory in the rats including delayed non-matching-to-place, delayed matching-to-sample, Morris water maze and Cincinnati water maze. The study in this thesis aimed to investigate the acute and long-term effect of MDMA on memory using the novel object

discrimination which tests recognition as well as working memory as these types of memory are impaired in MDMA users (see *section 1.7*).

The novel object discrimination is a recognition memory task involving an innate preference of the rats for novelty. The novel object discrimination protocol used in this thesis is modified from Ennaceur and Delacour (1988). The test consists of two trials; familiarisation and choice trials with varied inter-trial interval (Figure 1.10). In the familiarisation trial a rat is allowed to explore two identical objects. Following an inter-trial interval, one of the identical objects is replaced by a novel object in the choice trial. The normal rats spend more time exploring the novel object, suggesting that the familiar object is recognized (Dix and Aggleton, 1999, Ennaceur and Delacour, 1988). The novel object discrimination task is a 'pure' working memory task as it is completely free of reference memory and reinforcements such as electric shock or food (Ennaceur and Meliani, 1992). In addition advantages of novel object discrimination task, in comparison to other animal models for cognition, are that the novel object discrimination requires less training and does not induce high levels of arousal and stress, therefore it is more closely related to conditions under which human recognition memory is measured (Ennaceur and Delacour 1988).

Both 5-HT and dopamine neurotransmission, the neurotransmitters affected by MDMA, have been demonstrated to be involved in novel object discrimination. For example the 5-HT_{1A} receptor antagonist, WAY 100635 improved novel object recognition in rats (Pitsikas et al., 2003, Schiapparelli et al., 2006) while

the 5-HT_{1A} receptor agonist, 8-OH-DPAT caused impairment of novel object discrimination performance (Pitsikas et al., 2005). In addition the novel object discrimination was improved following administration of the 5-HT_4 receptor agonist, RS67333 and the 5-HT_6 receptor antagonists, SB271046 and Ro-046790 (King et al., 2004, Lamirault and Simon, 2001). Hotte et al (2005) found that dopamine D_1 receptor activation impaired object recognition with a short delay while improved object recognition after long delay. In addition Chuhan and Taukulis (2006) showed that methylphenidate, a dopamine and noradrenaline reuptake transporter inhibitor impaired novel object discrimination using a 24 h delay between trials.

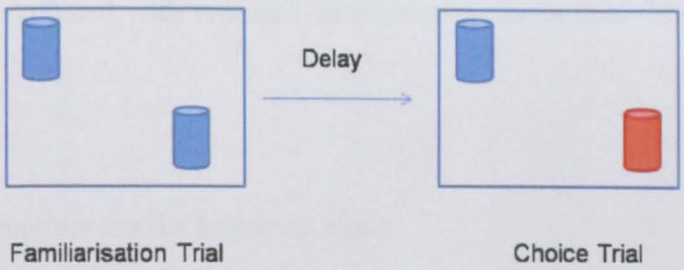


Figure 1.10 The novel object discrimination used in this thesis is modified from Ennaceur and Delacour (1988). The test comprises of two trials; familiarisation and choice trials. In the familiarisation trial two identical objects are presented to the rat followed by a delay. In the choice trial one of the identical objects is replaced by a novel object. The normal rats spend more time exploring the novel object as they recognise the familiar object.

1.9. Objectives

The review of the literature has shown that MDMA produces acute and long-term effects on various neurotransmitter systems especially 5-HT and dopamine, two of the neurotransmitter systems associated with learning and memory. Additionally there is a growing evidence for MDMA-induced learning and memory impairments in experimental animals and possibly humans. The interpretation of effects of MDMA in animals to humans is one of concern due to the fact that animal studies use high doses to produce neurotoxicity. Moreover the dosage regimens used in animal studies are often not comparable to human use patterns. This thesis focuses on using low doses of MDMA combined with repeated administration to imitate 'binge' use in human.

This thesis therefore has the following aims:

1. To determine the acute effects of single low doses of MDMA on working memory and changes in amine neurotransmitter levels relevant to the acute memory deficits

2. To determine the acute effects of repeated administration of low doses of MDMA, which imitates 'binge' use of MDMA in humans, on locomotor activity, body temperature and changes in extracellular 5-HT in the hippocampus

3. To determine the long-term effects of repeated low doses of MDMA on working memory and 5-HT neurotoxicity and investigate an influence of housing conditions during MDMA administration on MDMA-induced acute change in body temperature and consequent long-term 5-HT neurotoxicity

4. To determine the importance of tyrosine (and dopamine) depletion in MDMA-induced 5-HT neurotoxicity

CHAPTER 2

THE ACUTE EFFECT OF SINGLE LOW DOSES OF MDMA ON NOVEL OBJECT DISCRIMINATION

2.1. Introduction

There is growing evidence for a link between cognitive impairments and MDMA administration in humans and experimental animals (Morgan, 2000, Parrott, 2006, Piper, 2007). However the acute effects of MDMA on learning and memory in the rat have been relatively little investigated. Marston et al (1999) reported an acute disruption of the delayed non-matching to place when MDMA was given twice daily to rats over 3 days in ascending doses (10, 15 and 20 mg/kg). In addition when given a single low dose of MDMA (1, 2 or 3 mg/kg) to rats 20 min before the eight-arm radial maze task, Braida et al (2002) showed a modest effect of MDMA on short-term working memory and impairment of long-term working memory. The more recent study by Harper et al (2005) showed that MDMA (2 and 3 mg/kg) impaired the delayed matching-to-sample accuracy when given 10 min before the task. However this impairment was suggested to represent a disruption of attention rather than memory (Harper et al., 2005, 2006). To date there has not been a study investigating the involvement of specific neurotransmitters in the acute memory disruptions caused by MDMA.

The research described in this chapter aimed to determine the acute effects of a single low dose of MDMA on recognition memory using novel object discrimination and simultaneously to investigate changes in amine neurotransmitters in specific brain regions which are thought to be responsible for the cognitive disruption.

2.2. Materials and methods

2.2.1 Animals

Adult male Lister hooded rats (Biomedical Service Unit, University of Nottingham) weighing 250-350 g were housed in groups of 3-4 on a 12 h light/dark cycle (light on at 07.00 h) and given food and water *ad libitum*. Room temperature (21±2 °C) and humidity (45-65%) were kept constant. All experiments were performed in accordance with the UK Animals (Scientific Procedures) Act, 1986 under project license 40/2715 and approved by the University of Nottingham Local Ethical Review Committee.

Lister hooded rats were used in the present study because this strain of rat has good eyesight compared to albino rat strains due to the pigmented nature of hooded rats. This allows Lister hooded rats to perform better in a visual discrimination task such as novel object discrimination. Recently Ennaceur et al (2005) showed that male and female Lister hooded rats had better performance in novel object discrimination task compared to male Wistar rats while female Wistar rats cannot discriminate the novel from the familiar object. Dark Agouti rats were used in preference in the tyrosine depletion study (see Chapter 5), however it has poor performance in novel discrimination task as preliminary study showed that Dark Agouti rats cannot discriminate the novel from the familiar object in the choice trial of novel object discrimination with a 2-h inter trial interval. In addition Dark Agouti rats has less attention to the objects compared to Lister hooded rats as it was shown in the preliminary study that total time spent exploring the novel and familiar objects in the

choice trial of Dark Agouti rats was 8 ± 1 s compared to 34 ± 5 s of Lister hooded rats in this experiment.

2.2.2 Novel object discrimination

Novel object discrimination used in the present study was modified from (Ennaceur and Delacour, 1988). The apparatus comprised a clear Perspex box (39 x 23.5 cm with 30 cm high walls) as an arena and the objects to be discriminated were plastic bottles (8 cm high x 5 cm diameter) covered in white masking tape alone (familiar object) or white and black masking tape (novel object). Objects were cleaned with 20% v/v ethanol prior to each experiment to remove any olfactory cues, and each bottle was then inverted and secured with Blu-tack through holes in the floor located 5 cm from the side and 10 cm from the end walls in opposite corners (front left and back right) of the arena. The weight of each bottle was such that the rats could not displace it. Experiments were performed in constant light (200 Lux at floor level in the arena) between 10.00 and 14.00 h.

The experiment comprised of two consecutive trials; the first familiarisation trial (Trial 1; T1) and the second choice trial (Trial 2; T2). Twenty-four hours prior to testing, each rat was habituated to an individual test arena for 60 min in the absence of any object. On the test day, each group of rats received either MDMA (1 mg/kg or 3 mg/kg i.p., (±)-MDMA HCl, Sigma, UK) or saline (1 ml/kg i.p.). Thirty minutes later, each rat was habituated in the individual arena for 3 min in the absence of any object and then the rat was returned to its own home cage for 1 min. In the familiarisation trial rats were exposed to the two

identical objects for 3 min followed by a 2 h inter-trial interval. In previous studies at Nottingham it has been shown that Lister hooded rats can discriminate the novel from the familiar object with an inter-trial interval of up to 3 h (King et al., 2004). In the choice trial one of the identical objects was replaced with a bottle of identical size and shape but with horizontal black stripes (novel object). The remaining object from trial 1 was left untouched (familiar object). During both trials the exploration of either object was defined as the time spent sniffing, licking, chewing or touching it with the nose or within 1 cm of it with moving vibrissae and was recorded separately for each object by stopwatches. Sitting on the object was not regarded as exploratory activity.

Immediately after novel object discrimination rats were killed and the brains were quickly removed from the cranial cavity and dissection carried out on a chilled glass plate. The frontal cortex was cut 3 mm from the front of the brain. The hippocampus and striatum were dissected out and each half of these structures was kept separately in 1.5-ml eppendorf tubes. Brain regions were quickly frozen in liquid nitrogen immediately following dissection and stored at – 80 °C for later analysis of 5-HT, 5-HIAA, dopamine, DOPAC and HVA.

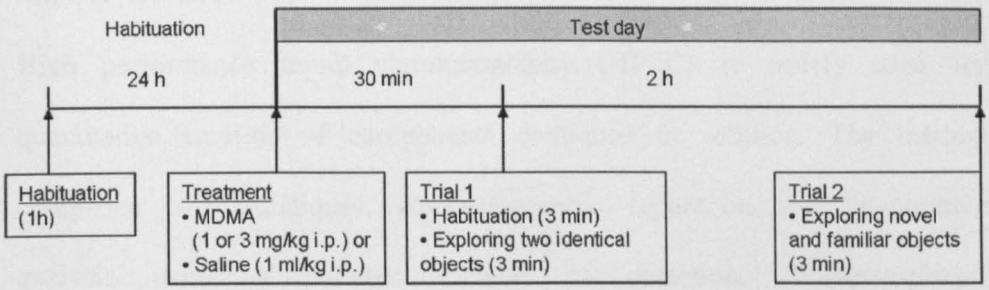


Figure 2.1 Diagrammatic representation of the experimental protocol

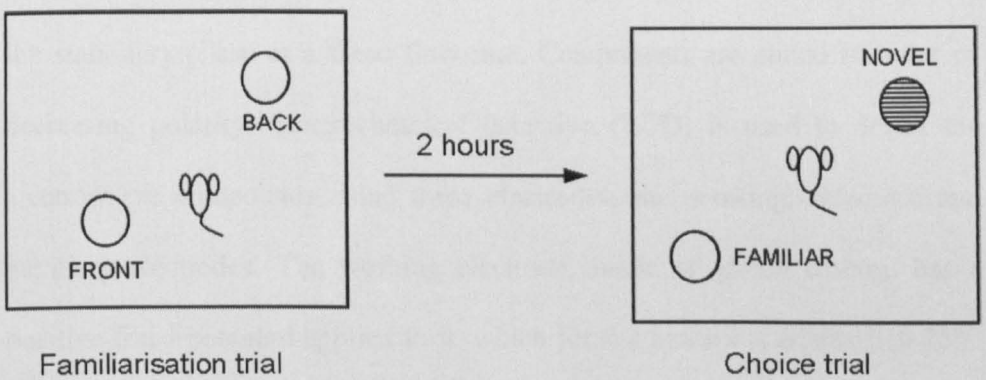


Figure 2.2 Diagram representing the familiarisation trial, comprised of two identical objects (bottle with white masking tape), and the test trial when one of the familiar objects (white masking tape) was replaced by a novel object (white and black masking tape).

2.2.3 Measurement of dopamine, 5-HT and their metabolites in brain tissues using HPLC-ECD

High performance liquid chromatography (HPLC) is widely used for quantitative analysis of components contained in solution. The method comprises two techniques; chromatographic separation and quantitative analysis using a suitable method of detection. "Reverse-phase" chromatography is generally used for measurement of monoamine neurotransmitters. The stationary phase consists of alkyl silylating reagent bound to the silica particles which create a hydrophobic, non-polar surface. The polar mobile phase, consisting of water and methanol, was pumped over the stationary phase at a fixed flow rate. Components are eluted in order of decreasing polarity. Electrochemical detection (ECD) is used to detect the electroactive compounds using three electrodes; the working, reference and auxiliary electrodes. The working electrode, made of glassy carbon, has a positive fixed potential applied to it, which for the amines is normally 0.75V. The reference electrode usually consists of a silver/silver chloride (Ag/AgCl) maintained at a fixed the potential. The components in the samples are separated by a silica column (according to molecular mass and charge in solution) and then pass through the cell, over the working electrode where the molecules are oxidized and electrons transferred to it thereby generating a current which is directly proportional to the amount of electroactive substance present. The current is amplified and converted into a signal which is recorded on a chart recorder or chromatography integrator. The potential is governed by applied potential at which the molecules undergo the redox reaction underlying this method (Figure 2.3).

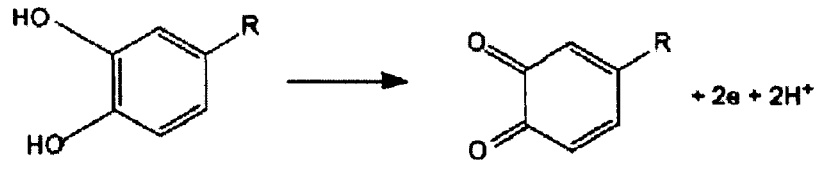
Tissue sample preparation

Tissue samples were weighed and then homogenized with 1 ml ice-cold solution of 0.1 M perchloric acid containing 0.1 % w/v sodium metabisulfite and 0.01 % w/v disodiumethylenediamine-tetraacetate (EDTA) using a sonicator (Soniprobe, output ~20, 20-30 s). The homogenate was centrifuged at 17500 g for 20 min at 4 °C (Harrier 18/80 refrigerated, MSE). The supernatant was filtered and then kept at -80°C for later analysis.

HPLC-ECD

The HPLC system consisted of a Jasco PU-980 pump, Rheodyne 7125 injection valve and SP4290 integrator. The mobile phase; 0.05 M potassium dihydrogen orthophosphate (KH_2PO_4), 0.1 mM EDTA, 0.16 mM octane sulfonic acid (disodium) and 13.5% methanol dissolved in HPLC grade water, pH adjusted to 3.0 with *o*-phosphoric acid, pumped at 1 ml/min through SphereClone column (C18, 4.6 x 150 mm, 5µm; Phenomenex, Macclesfield, GB). A 20-µl of 10^{-7} M mixed standard of 5-HT, 5-HIAA, dopamine, DOPAC and HVA was routinely injected onto the column. Quantification was achieved using Antec CU-04 controller and Antec VT-03 cell fitted with a glassy working carbon electrode (Antec Leyden, Zoeterwoude, The Netherlands) with the potential set at 0.75V versus Ag/AgCl reference electrode. The lower limit of detection was 0.1 pmol injected onto the column. The internal standard was not used in the present study as the process of tissue sample preparation is simple and does not involve multiple process of extraction. The internal standard used in the analysis for 5-HT, 5-HIAA, noradrenaline and dopamine concentrations in brain tissue is *N*-methyl-5-HT (Durkin et al., 2008).

Catecholamine



Indoleamine

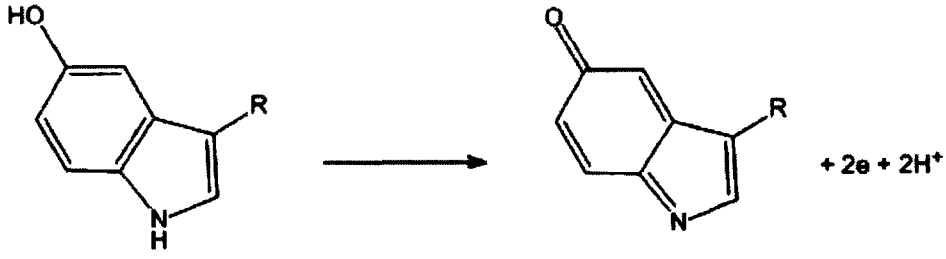


Figure 2.3 Diagram showing the oxidation of catecholamine and indoleamine that occurs at the surface of the carbon electrode.

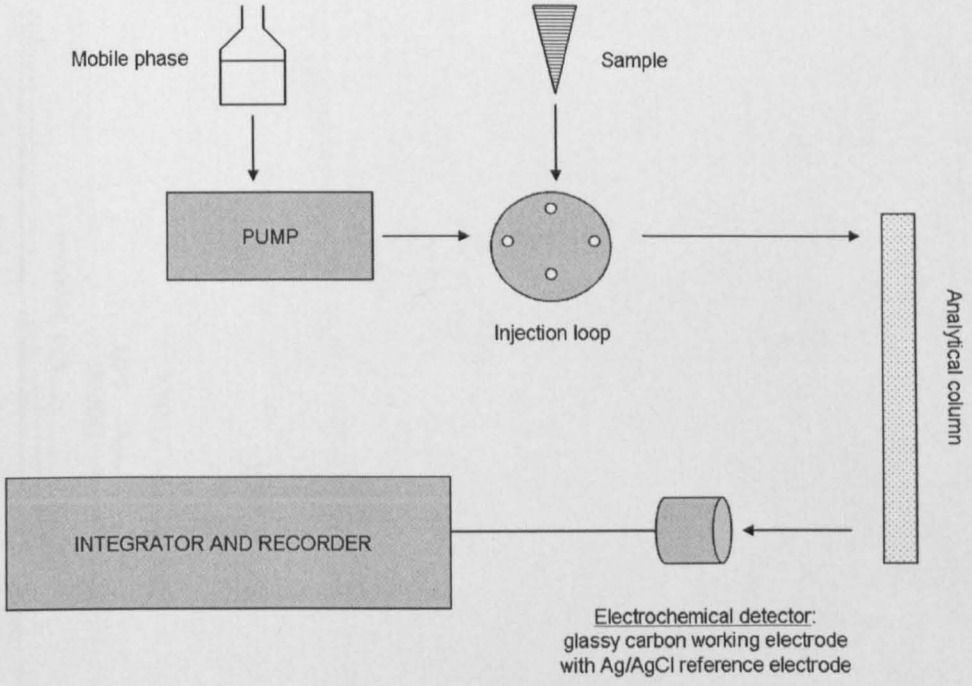


Figure 2.4 The components of HPLC-ECD system used in this thesis

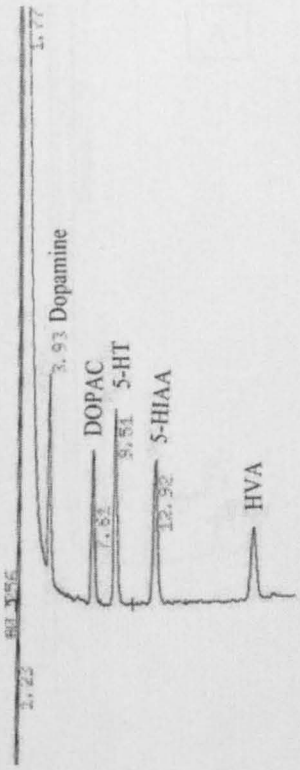


Figure 2.5 An example of the chromatographic separation of dopamine, DOPAC, 5-HT, 5-HIAA and HVA (10^{-7} M each) in the mixed standard using mobile phase 0.05 M KH_2PO_4, 0.1 mM EDTA, 0.16 mM octane sulfonic acid and 13.5 % v/v methanol pH 3.0.

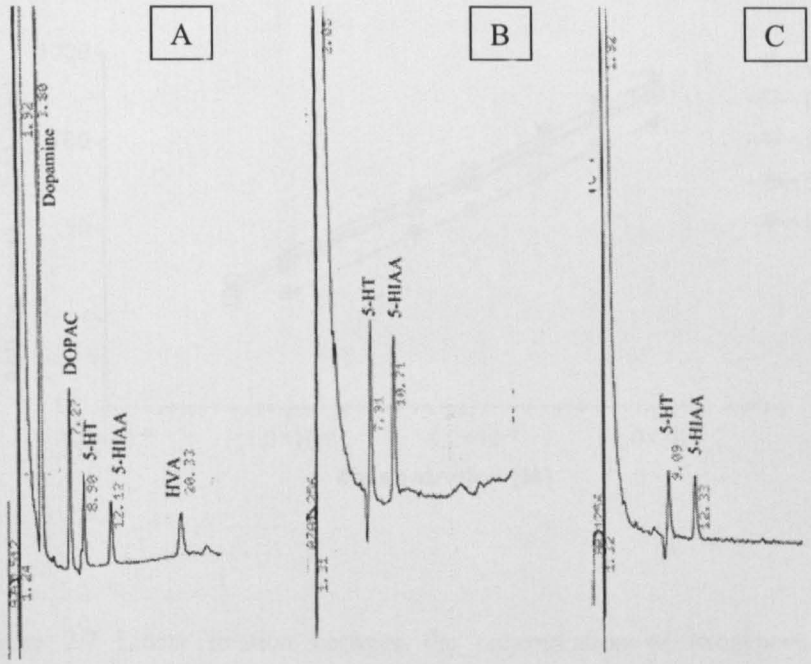


Figure 2.6 Examples of chromatograms from striatum (A) hippocampus (B) and frontal cortex (C). Tissue samples were weighed and then homogenized with 1 ml ice-cold solution of 0.1 M perchloric acid containing 0.1 % w/v sodium metabisulfite and 0.01 % w/v EDTA using a sonicator (Soniprobe, output ~20, 20-30 s). The homogenate was centrifuged at 17500 g for 20 min at 4 °C (Harrier 18/80 refrigerated, MSE). The supernatant was filtered and then kept at -80 °C for later analysis. There are dopamine, DOPAC, 5-HT, 5-HIAA and HVA peaks in the striatum sample (A) while there are 5-HT and 5-HIAA peaks in the hippocampus (B) and frontal cortex (C) samples.

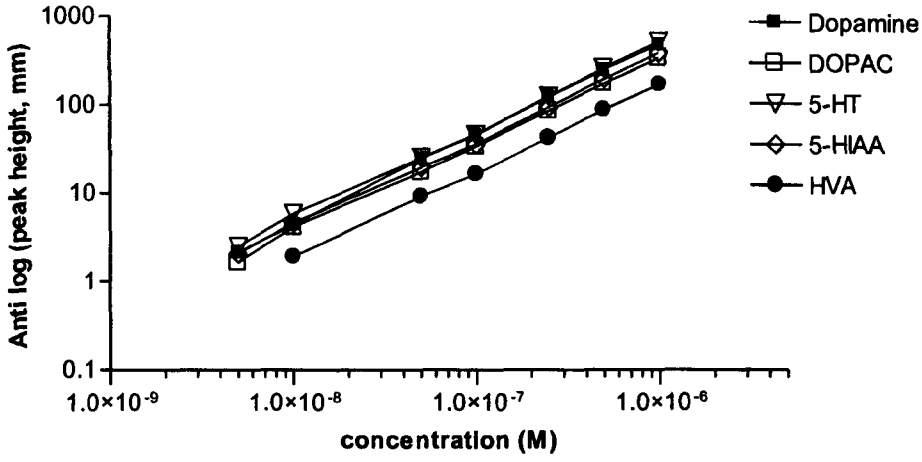


Figure 2.7 Linear relation between the concentration of exogenous dopamine, DOPAC, HVA, 5-HT and 5-HIAA injected onto the column and the detector response.

2.2.4 Statistical analysis

For novel object discrimination, data are presented as time (s) spent exploring each of the identical objects during the familiarisation trial, and time (s) spent exploring the novel object versus the familiar object during the choice trial (± SEM) and two-way ANOVA was used to analyse overall effect of treatment and object on time spent exploring the objects followed by Bonferroni *post hoc* test for within-group comparisons of time spent exploring each object. The exploratory data in the choice trial was converted to a mean preference index (PI). PI is a measure of the percentage time spent exploring the novel object compared to the total time exploring both objects in the choice trial (e.g. [novel object/ novel object + familiar object] x 100) (Bruel-Jungerman et al., 2005). The PI of each group of rats was then compared to a PI value of 50% chance level performance using one sample *t*-test. Between-group comparison of total time that rats spent exploring two identical objects in the familiarisation trial (total T1 time) and the novel and the familiar object in the choice trial (total T2 time) (s) were assessed using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. A significant difference was considered at $p<0.05$.

For amine neurotransmitters analysis in the striatum, hippocampus and frontal cortex, between-group comparison of amine neurotransmitter concentrations (pmol/mg of tissue) was made using one-way ANOVA followed by Tukey's *post hoc* test. A significant difference was considered at $p < 0.05$.

2.3. Results

2.3.1 The acute effect of low doses of MDMA on novel object discrimination

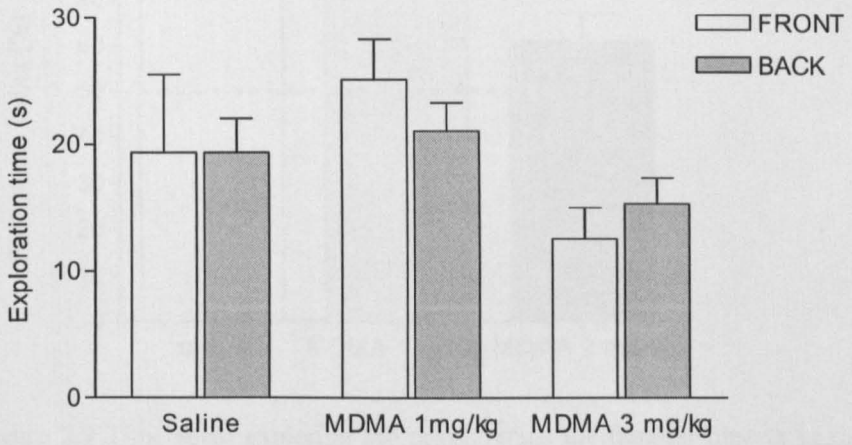
To determine the acute effect of low doses of MDMA on novel object discrimination rats were given either MDMA (1 mg/kg or 3 mg/kg) or saline 30 min prior to the familiarisation trial followed by 2 h inter-trial interval before the choice trial. In the familiarisation trial when the rats were exposed to two identical objects, all three groups of rats showed no difference in time exploring the two identical objects (Figure 2.8A). In the choice trial which was 2 h after the familiarisation trial, two-way ANOVA revealed an overall effect of the objects ($F(1,25) = 31.91$, $p < 0.0001$) on time spent exploring the novel versus familiar object, such that Bonferroni *post hoc* test showed that rats in the control group (saline injected) spent a significantly greater time exploring the novel versus the familiar object ($p < 0.01$), indicating an ability to discriminate between the novel and familiar objects. This ability to discriminate was also seen in the rats treated with MDMA (1 mg/kg) as they spent significantly more time exploring the novel rather than the familiar object ($p < 0.01$) in trial 2 (Figure 2.8B). However, the higher dose of MDMA (3 mg/kg) caused an impairment in novel object discrimination in trial 2, as the rats spent equivalent times exploring the novel and the familiar objects ($p > 0.05$; Bonferroni *post hoc* test) (Figure 2.8B).

Object exploratory times in the choice trial were converted into the preference index (PI) shown in Figure 2.9. The PI value of either MDMA (1 or 3 mg/kg) treated rats was not significantly different from saline-treated rats ($p > 0.05$,

unpaired Student *t*-test). However the PI values of saline and MDMA (1 mg/kg) treated rats were significantly greater than the PI value of 50% chance level ($p = 0.0002$ and $p = 0.0004$ respectively, one sample *t*-test) while the PI value of MDMA (3 mg/kg) treated rats was not significantly different from 50% chance level ($p = 0.1105$, one sample *t*-test) (Figure 2.9).

The total time that the rats spent exploring the objects in trial 1 (total T1 time) and trial 2 (total T2 time) are shown in Table 2.1. Total T1 time varied between groups of rats but the differences were not significant. The data showed that total T1 time in the MDMA (1 mg/kg)-treated group (47 ± 4 s) was higher than that of the saline-treated rats (39 ± 7 s) while total T1 time of the MDMA (3 mg/kg)-treated rats (28 ± 4 s) was the lowest. However, there was no difference in the total time spent exploring the novel and the familiar object in trial 2 (one-way ANOVA).

(A)



(B)

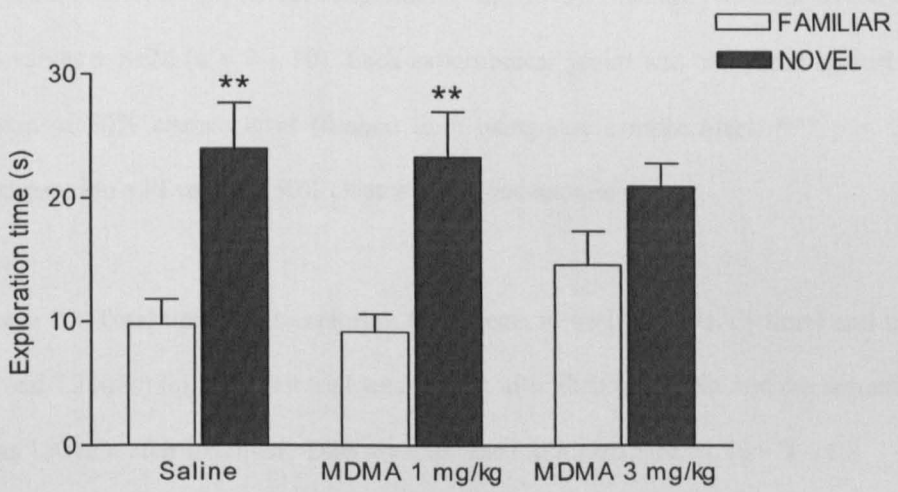


Figure 2.8 Effects of MDMA (1 and 3 mg/kg i.p.) on the time spent (s, means ± SEM) exploring each of the identical objects during trial 1 (A) and the novel versus the familiar object during trial 2 which was 2 h after trial 1 (B). The first trial was 30 min after drug treatment and the second trial was 150 min after treatment. ** $p < 0.01$ compared with time spent at the familiar object in the same treatment group, Bonferroni *post hoc* test ($n = 9 – 10$).

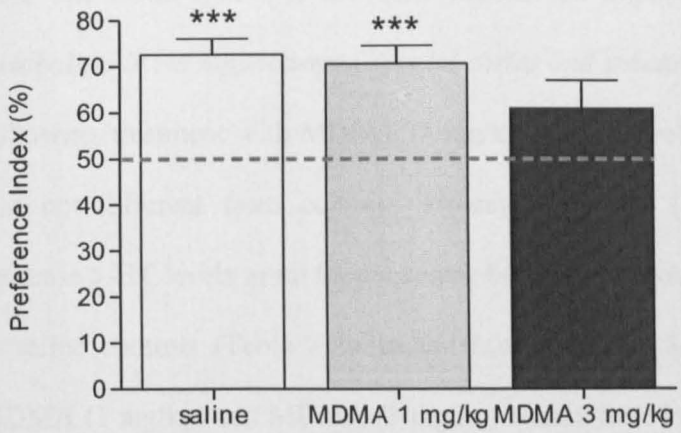


Figure 2.9 Time spent exploring the novel versus the familiar objects in the choice trial was converted into a preference index (PI) (e.g. [novel object/ novel object + familiar object] x 100) (Bruel-Jungerman et al., 2005). Data are presented as means of PI values ± SEM (n = 9 – 10). Each experimental group was compared against a PI value of 50% chance level (dashed line) using one sample *t*-test. *** $p < 0.001$ compared to a PI value of 50% chance level (one-sample *t*-test)

Table 2.1 Total time spent exploring the objects in trial 1 (Total T1 time) and trial 2 (Total T2 time) (s). The first trial was 30 min after drug treatment and the second trial was 150 min after treatment. Data are expressed as means ± SEM. (n = 9 – 10)

Treatment	Total T1 time (s, mean ± SEM)	Total T2 time (s, mean ± SEM)
saline	39 ± 7	34 ± 5
MDMA (1 mg/kg)	46 ± 4	33 ± 4
MDMA (3 mg/kg)	28 ± 4	36 ± 3

2.3.2 The acute effects of low dose MDMA on dopamine, 5-HT and their metabolites in rat hippocampus, frontal cortex and striatum

Following treatment with MDMA (1 mg/kg), 5-HT levels in rat hippocampus was not different from controls. However MDMA (3 mg/kg) tended to decrease 5-HT levels in rat hippocampus but this was not significant compared to saline controls (Table 2.2). In the frontal cortex, 5-HT levels following MDMA (1 mg/kg) and MDMA (3 mg/kg) were a little lower than controls but again this was not significant (Table 2.2). In the striatum, there was no significant difference between 5-HT levels following saline and MDMA (1 mg/kg and 3 mg/kg) (Table 2.2).

5-HIAA, the major 5-HT metabolite, was also measured in the hippocampus, frontal cortex and striatum 150 min after MDMA (1 and 3 mg/kg) administration. Data showed no significant different in 5-HIAA levels in the hippocampus between saline and MDMA (1 and 3 mg/kg) treated rats (Table 2.2). Similar lack of drug treatment was seen in the frontal cortex and the striatum (Table 2.2).

Dopamine and its metabolites, DOPAC and HVA, concentrations were measured only in the striatum. The results show that dopamine, DOPAC and HVA in the striatum of MDMA (1 and 3 mg/kg) treated rats were not significantly different from saline controls (Table 2.3).

Table 2.2 5-HT and 5-HIAA levels (pmol/mg tissue) in rat hippocampus, frontal cortex and striatum 150 min after MDMA (1 or 3 mg/kg i.p.) or saline (1 ml/kg i.p.). Data are presented as means ± SEM. (n = 8 - 10)

	Hippocampus		Frontal cortex		Striatum	
Treatment	5-HT	5-HIAA	5-HT	5-HIAA	5-HT	5-HIAA
Saline	2.34±0.13	2.59±0.26	2.67±0.18	1.67±0.24	3.08±0.23	2.99±0.19
MDMA (1 mg/kg)	2.45±0.25	2.49±0.10	2.52±0.15	1.64±0.28	3.04±0.12	2.85±0.11
MDMA (3 mg/kg)	2.14±0.19	2.51±0.42	2.53±0.13	1.43±0.19	3.25±0.20	2.69±0.20

Table 2.3 Dopamine and its metabolites, DOPAC and HVA levels in rat striatum 150 min after MDMA (1 or 3 mg/kg i.p.) or saline (1 ml/kg i.p.). Data are presented as means ± SEM. (n = 8 – 10)

Treatment	Dopamine	DOPAC	HVA
saline	35.56 ± 5.26	5.42 ± 1.06	3.15 ± 0.57
MDMA (1 mg/kg)	41.28 ± 4.21	5.63 ± 0.69	3.28 ± 0.30
MDMA (3 mg/kg)	38.84 ± 1.86	5.69 ± 0.66	3.27 ± 0.30

2.4. Discussion

This chapter reviews studies examining the acute effect of single low doses of MDMA on memory assessed by using novel object discrimination in the adult rat. The MDMA doses used in this study (1 and 3 mg/kg) were lower than the doses of MDMA used to induce serotonergic neurotoxicity in rats (10-40 mg/kg) (Battaglia et al., 1987, Commins et al., 1987, Schmidt et al., 1987). In addition, the doses selected for this study more closely represent the equivalent dose for occasional use of the drug by humans as reviewed recently (Green et al., 2009).

The main finding was that acute injection of 3 but not 1 mg/kg MDMA disrupted novel object discrimination without altering brain 5-HT and 5-HIAA levels. The finding of memory disruption following a low dose MDMA administration in the present study is in agreement with studies demonstrated by Braida et al (2002) and Harper et al (2005) who found acute memory impairments in eight-arm radial maze and delayed matching-to-sample (DMTS) tasks respectively following single low doses of MDMA (1-3 mg/kg).

The impairment of novel object discrimination presented in this study however cannot be solely interpreted as a specific disruption of cognitive function as it is noticeable that MDMA (3 mg/kg)-treated rats have less total exploratory activity in the familiarisation trial, which was 30 min after drug administration than the other two groups (Table 2.1). A decrease of exploratory activity in the familiarisation trial following MDMA (3 mg/kg) might be due to MDMA

causing either less attention, or a direct alteration of locomotor activity and stereotype related to induction of components of the serotonin syndrome observed with drug induced release of 5-HT. Harper et al (2005) suggested that the acute impairment of an overall delay-independent DMTS accuracy following MDMA reflected an impairment of the attention process rather than memory. In addition MDMA-induced acute 5-HT release causes hyperactivity and the serotonin syndrome (Callaway et al., 1990, Spanos and Yamamoto, 1989) and this might disrupt normal exploratory activity. However, the study by Braida et al (2002) there was no significant change in locomotor and stereotype activity after 20 min of acute low doses of MDMA (1-3 mg/kg i.p.). Similarly in the present study there was no change in stereotypies observed in MDMA-treated rats. In summary less exploration of the identical objects in the familiarisation trial in MDMA (3mg/kg)-treated rats may result in less memory for the familiar object and thus influence recognition in the choice trial.

The neurochemical data showed no change in dopamine, 5-HT and their metabolites in the hippocampus, frontal cortex and striatum 150 min after either doses of MDMA. The main limitation of the present study was the lack of a neurochemical profile during the novel object discrimination task thus it cannot be determined whether the impairment of novel object discrimination involved an acute change in neurotransmitter function (such as 5-HT and dopamine). In a comparable study by Gudelsky and Nash (1996), using *in vivo* microdialysis, MDMA (2.5 mg/kg) produced a significant increase of extracellular 5-HT concentration 30 to 90 min after drug injection in the striatum and 30 to 60 min after drug injection in the median prefrontal cortex

5-HT levels returned to the baseline 150 min following MDMA (2.5 mg/kg) administration (Gudelsky and Nash, 1996). In addition a study by Baumann et al (2008) again using *in vivo* microdialysis showed acute dopamine release in the striatum and prefrontal cortex following two injections of MDMA (1 and 3 mg/kg) at 1 h intervals. As 5-HT and dopamine have been suggested to be important in learning and memory (see review Floresco and Magyar, 2006, King et al., 2008, Meneses, 2003, Missale et al., 1998), this profile of 5-HT and dopamine release following low doses of MDMA suggests that the behavioural change during novel object discrimination in the present study may relate to changes in 5-HT and dopamine release.

In conclusion, the present results add to the growing evidence of acute disruption of learning and memory processes following low dose administration of MDMA (3 mg/kg) in the rat. The limitation of the present study was clearly the lack of functional and neurochemical measurements during the behaviour as mentioned above. To address this issue in the next chapter the intracerebral microdialysis technique was used to measure the acute changes in 5-HT concomitant with behavioural assessment in response to systemic administration of low doses of MDMA.

CHAPTER 3

THE ACUTE EFFECTS OF REPEATED ADMINISTRATION OF LOW DOSES OF MDMA ON LOCOMOTION, BODY TEMPERATURE AND EXTRACELLULAR 5-HT IN THE HIPPOCAMPUS

3.1. Introduction

Over the last decade, the pattern of MDMA use in humans has been often characterized as repeated drug administration on a single occasion and referred to as 'binge use' (Hammersley et al., 1999, Parrott, 2005, Topp et al., 1999, Winstock et al., 2001). It is claimed that binge use of MDMA boosts the subjective effects of MDMA possibly by reducing tolerance effects of repeated usage (Parrott, 2005). This pattern of use may lead to an increased risk of acute and long-term toxicity. Pharmacokinetic studies in humans showed that repeated administration of MDMA presented non-linear pharmacokinetics (de la Torre et al., 2000) as MDMA is a CYP 2D6 enzyme inhibitor which thus causes an inhibition of its own metabolism (Delaforge et al., 1999). Consequently Farre et al (2004) reported an increase of area under the plasma concentration-time curve (AUC) and maximum plasma drug concentrations (C_{max}) of MDMA after a second dose following a 24 h interval and increased pharmacological effects such as increase in blood pressure, heart rate, subjective effects and cortisol levels. Similar to the effects in humans repeated administration of MDMA (3 and 9 mg/kg twice daily for 4 days) increased mean arterial blood pressure, produced biphasic (decrease and increase) heart rate and produced cardiac toxicity in rats (Badon et al., 2002).

The hyperthermic response is a well established severe acute adverse effect of MDMA ingestion (Green et al., 2003, Liechti and Vollenweider, 2000). It can lead to other associated clinical problems including rhabdomyolysis,

intravascular coagulation and acute renal failure (Green et al., 2003). In animal studies Green et al (2004) reported a dose-dependent MDMA-induced hyperthermia following repeated MDMA (2, 4 and 6 mg/kg x 3 every 3 h) to the rats. Baumann et al (2008a) also showed a marked and sustained increase of body temperature following MDMA (7.5 mg/kg x 3 every 2 h) administered to rats. In addition to acute hyperthermia induced by repeated administration of MDMA, Kindlundh-Hogberg et al (2007) reported hyperactivity caused by repeated MDMA (5 mg/kg x 3 every 3 h). This pattern of MDMA administration also enhanced activity in the centre of the open field arena indicating reduced anxiety or enhanced impulsivity which is known to be associated with altered 5-HT activity (Kindlundh-Hogberg et al., 2007).

Although evidence for acute effects of repeat administration of MDMA have been reported, no study has looked at acute functional changes, such as activity and body temperature, together with changes in extraneuronal neurotransmitter levels. The aim of the research in this chapter was to determine the acute effects of repeated low doses of MDMA to imitate binge use in humans on locomotor activity, body temperature and extracellular 5-HT in the hippocampus by combining the techniques of radiotelemetry and brain microdialysis so that body temperature, activity and 5-HT release in the hippocampus could be measured simultaneously in the same animal. The second aim was to examine whether changes in locomotor activity or body temperature correlated with changes in extracellular 5-HT in the hippocampus.

3.2. Materials and methods

3.2.1 Animals

Male Lister-hooded rats (Biomedical Services Unit, University of Nottingham, UK) were kept at a constant ambient temperature (21±2 °C) and humidity (45-65 %) on a 12 h light/dark cycle (light on at 07.00 h) with free access to food and water. All experiments were performed in accordance with the UK animals (Scientific Procedures) Act 1986 and approved by the University of Nottingham Local Ethical Review Committee.

3.2.2 Experimental procedure

Rats weighing 100-130 g were implanted with a radiotransmitter into the peritoneal cavity under isoflurane anaesthesia and then allowed to recover in an individual cage for 1 week. Seven days after surgery each individual cage was moved to the telemetry room and placed over a receiver where the rats were monitored for their normal activity and body temperature for 1 week. In the telemetry room temperature (21±1 °C) and humidity (45-65 %) were kept constant. Food and water were available *ad libitum*. Fourteen day after radiotransmitter implantation, the rat was implanted with a microdialysis probe into the hippocampus under isoflurane anaesthesia and then allowed to recover overnight. The following day each rat received either MDMA (3 or 6 mg/kg) or saline (1 ml/kg) i.p. 3 times every 2 h (MDMA, synthesized by Department of Chemistry, University College Dublin, Ireland). Microdialysis samples were collected 1 h before the first injection until 2 h after the last injection. Body

temperature and locomotor activity were simultaneously recorded during treatment using radiotelemetry.

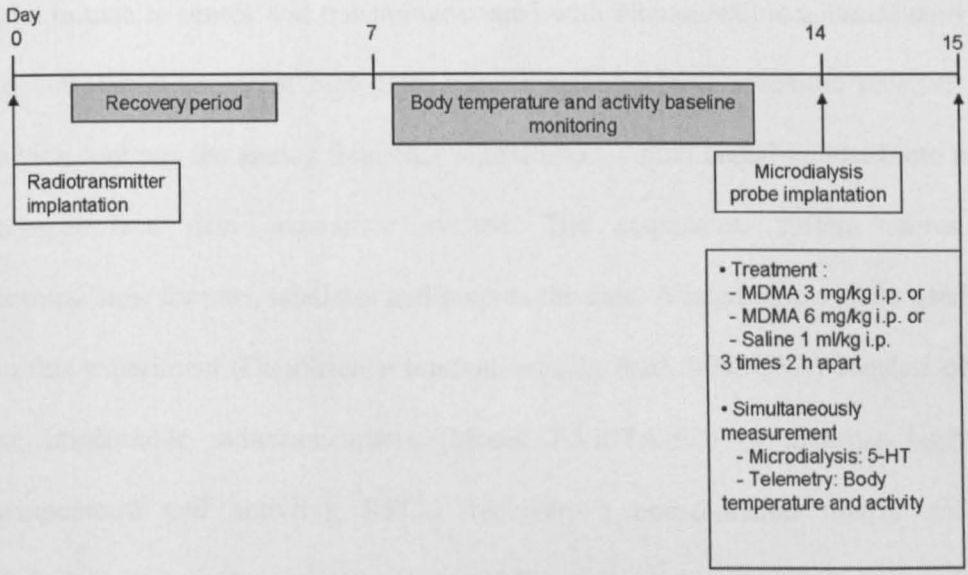


Figure 3.1 Diagrammatic representation of the experimental protocol.

3.2.3 Radiotelemetry

The radiotelemetric technique provides a tool to monitor physiological functions in awake and freely moving laboratory animals. The system consists of a miniature sensor and transmitter coated with biocompatible material used to detect and broadcast biological signals in animals to a remote receiver, which converts the analog frequency signal into a digital signal inputted into a computerized data acquisition system. The acquisition system stores, manipulates, formats, tabulates and outputs the data. A telemetric system used in this experiment (DataScience International, St Paul, MN, USA) consists of an implantable radiotransmitters (Model TA10TA-F20 to measure body temperature and activity), RPC-1 receiver, a consolidation matrix and A.R.T.v2.1 acquisition software (Figure 3.2).

The advantages of radiotelemetry in psychopharmacological research include reducing animal distress compared to conventional techniques, eliminating animal restraint, reducing the number of animals used by increasing the number of parameters that can be collected from a group of animals and permitting virtually unrestricted continuous data collection (Kramer and Kinter, 2003). Since radiotelemetry permits investigation of drug effects in freely moving unrestrained animals, it facilitates an animal model that mimics the clinical conditions of drug investigation.

Surgical procedure

A radiotransmitter (TA10TA-F20) was implanted into the peritoneal cavity of the rat under isoflurane general anaesthesia. Anaesthesia was induced using isoflurane (4%) in O_2:N_2O 1:2 and maintained using isoflurane (2.5%). During surgery the rat was placed on a heat pad to maintain body temperature. A small abdominal incision was made vertically at approximately 1 cm to an abdominal midline. A sterile radiotransmitter was inserted then subcutaneously sutured with absorbable suture (Fluorosorb) and skin closed by sterile clips which were removed 5 days after surgery. Analgesia (Rimadyl), local anaesthesic (Lignol) and fluid replacement (0.9% NaCl) were given and plastic wound dressing (Nobecutane, Astra Pharmaceutical) applied. Rat was allowed to recover in an individual home cage for 1 week before starting radiotelemetry recording.

Data manipulation and statistical analysis

When an individual cage was placed over the receiver, locomotor activity and body temperature were continuously sampled 10s every 2 min using A.R.T.v2.1 acquisition software. The data during treatment were calculated as means of activity (counts/min) and body temperature (°C) in 20-min time bins for individual rats thereby enabling a mean ± SEM value for each group at each time point to be calculated. In addition a calculation of the overall changes over the observation period of 120 min after each injection (area under the curve; AUC) was performed for locomotor activity. This allows a comparison of the size of response in each group as presented graphically in the results section. Data from changes in activity and body temperature in 20-min time bins and the AUC were analysed using repeated, 2-way ANOVA with treatment and

time as main factors followed by Bonferroni *post hoc* test. The significant difference was considered at $p < 0.05$. Differences were deemed significant at $p < 0.05$.

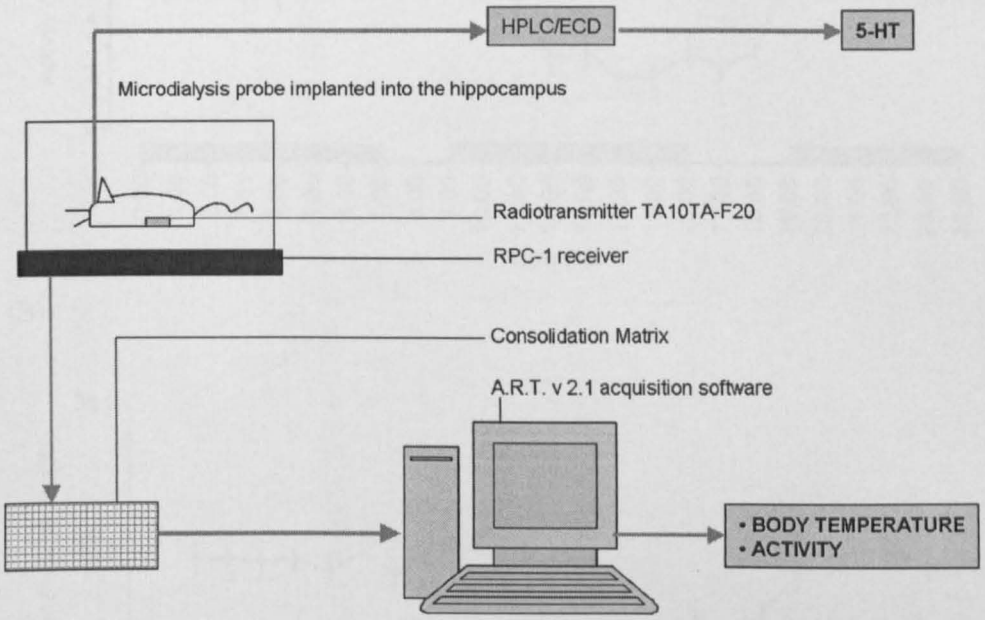
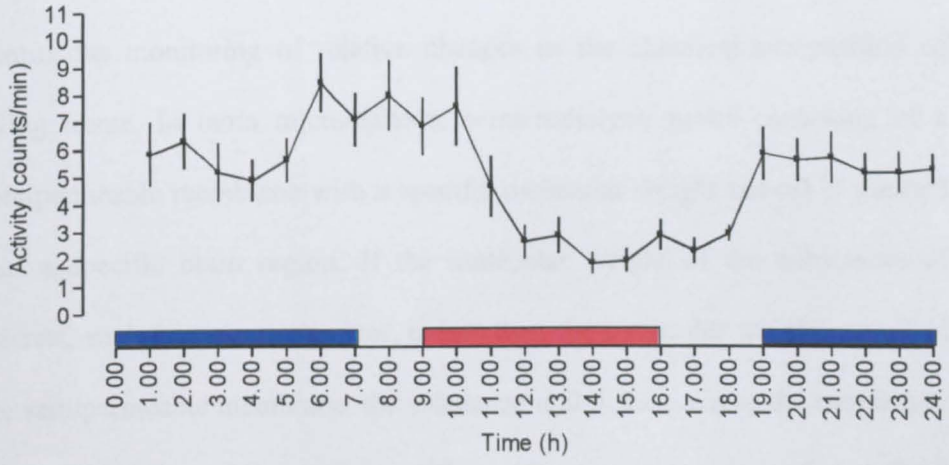


Figure 3.2 Combining radiotelemetry and microdialysis to simultaneously record locomotor activity, body temperature and 5-HT release in the hippocampus during administration of repeated low doses of MDMA. The telemetric system used in the present study (DataScience International, St Paul, MN, USA) consists of a radiotransmitter (TA10TA-F20) implanted into the peritoneal cavity of the rat; detecting body temperature and activity, RPC-1 receiver, a consolidation matrix and A.R.T. v2.1 acquisition software. A microdialysis probe was also implanted to the rat hippocampus under anaesthesia. Microdialysis samples were collected every 20 min then later analysis for 5-HT using HPLC-ECD.

(A)



(B)

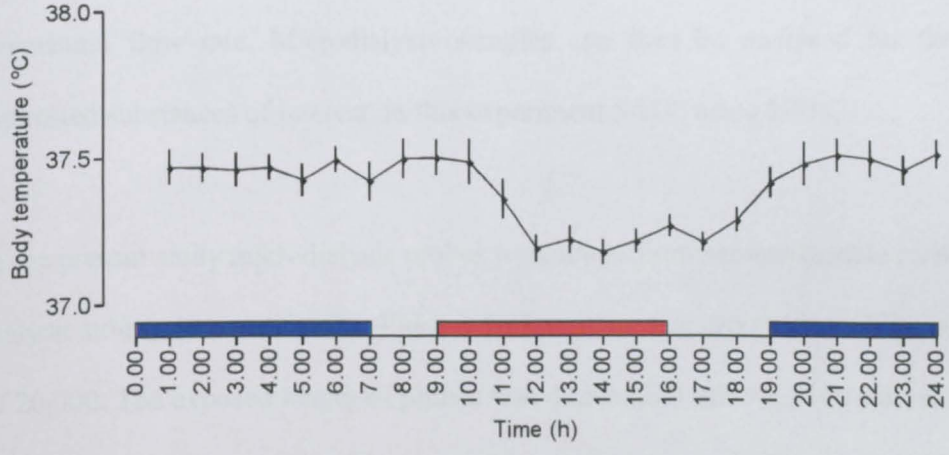


Figure 3.3 Radiotelemetry data for baseline monitoring of **A:** locomotor activity (counts/min) and **B:** body temperature (°C). The sample data selected were from day 4 after individual rat cages were placed over receivers in the telemetry room. Data are presented as mean in 1-h bins over 24 hours ± SEM (n = 18). The blue bar ( ) shows when the light is off (light off at 19.00h and on at 07.00h) and the red bar ( ) shows the experimental period on treatment day (09.00 - 16.00 h). Locomotor activity and body temperature were higher during the dark phase compared to those during the light phase.

3.2.4 Brain microdialysis

Microdialysis is an *in vivo* bioanalytical sampling technique which allows continuous monitoring of relative changes in the chemical composition of living tissue. In brain microdialysis, a microdialysis probe consisting of a semipermeable membrane with a specific molecular weight cut-off is inserted into a specific brain region. If the molecular weight of the substances of interest, such as neurotransmitter, is less than the molecular weight cut-off of the semipermeable membrane, the substance will diffuse across the membrane. The fluid balance of living tissue will not alter as an isotonic perfusion fluid, for example artificial cerebrospinal fluid (aCSF) for brain tissue, is perfused at a constant flow rate. Microdialysis samples can then be analysed for the interested substances of interest, in this experiment 5-HT, using HPLC.

In the present study microdialysis probes were made from semipermeable renal dialysis tubing (reconstituted cellulose) with a molecular weight cut off point of 20,000. The exposed length of probes was 4 mm (220 μm o.d., 180 μm i.d.) (Figure 3.4). The microdialysis probe was connected to a Harvard Microinfusion pump (Harvard Scientific, USA) via the liquid swivel system (Harvard Scientific, USA) to allow the animal relatively unrestricted movement (Figure 3.6). The artificial cerebrospinal fluid (aCSF) (in mM: NaCl 125.0, $NaHCO_3$ 27.0. KCl 2.5, NaH_2PO_4 0.5 Na_2HPO_4 1.2, $NaSO_4$ 0.5, $MgCl_2$ 1.0, $CaCl_2.2H_2O$ 1.0 adjusted to pH 7.4 with phosphoric acid) was constantly perfused through the probe at flow rate of 1.6 μl/min. To determine *in vitro* recovery, microdialysis probes were immersed in a 10^{-7} M solution of dopamine, DOPAC, 5-HT, 5-HIAA and HVA and perfused with aCSF at 1.6

μl/min then the microdialysis samples were analysed using the HPLC-ECD. An internal standard is not required for this experiment as there is no extraction process but direct measurement of 5-HT in the microdialysis samples. The recovery of 5-HT through the probe was 8%.

Surgical procedure

The microdialysis probe was implanted into the hippocampus under general anaesthesia as described in *3.2.3*. The rat was placed on the stereotaxic frame and fitted with ear bars. The rat skull was exposed and bregma located. The coordinates of the right hippocampus are AP -5.6, ML +4.6 relative to bregma and DV -8.0 below the dura using the rat brain atlas (Paxinos and Watson, 1998). At the coordinate, a hole was drilled through the skull and the probe lowered to the hippocampus. The probe was fixed to the skull with two skull screws and dental cements. The open incision was sutured and local anaesthesia (Lignol) applied before spraying with plastic wound dressing (Nobecutane, Astra Pharmaceutical). Rats were allowed to recover in the recovery box for at least 6 h and food pallets were replaced by moistened mash to help recovery. After the recovery period, rats were moved to the telemetry room. The recovery boxes were placed over the individual telemetry receiver. Rat activity and body temperature were monitored overnight before treatment and sample collection.

Sample Collection

On the following day, microdialysis samples were directly collected into the 0.1-ml HPLC-inserts vials (Kinesis, Bolnhurst, UK) every 20-min. After each collection the inserts were put into 1.5-ml eppendorf tubes and immediately frozen in liquid nitrogen. The first 3 samples in the first hour before treatment were calculated as an individual baseline. Immediately after the third collection rats were given either MDMA (3 or 6 mg/kg) or saline (1 ml/kg) i.p. 3 times every 2 h. Microdialysis samples were continuously collected every 20 min for 2 h after the final dose. Rat activity and body temperature data were simultaneously recorded during treatment using radiotelemetry.

Histology

The probe placement was confirmed in all rats at the end of the experiment. Only the microdialysis samples from rats in which the probe was located in the hippocampus were used for later analysis of 5-HT. To stain the position of the probe dye was injected through microdialysis probe. The brain was carefully removed and stored in paraformaldehyde solution (PFA) at 4 °C. Brains were then sliced into 20 μm sections. The section with the probe staining was placed on a slide under a light microscope and photographed.

Figure 3.5B shows a brain slice with a stained microdialysis probe in the hippocampus. There were 2 out of 20 probes were excluded as they were found to be incorrectly placed.

(A) (B)

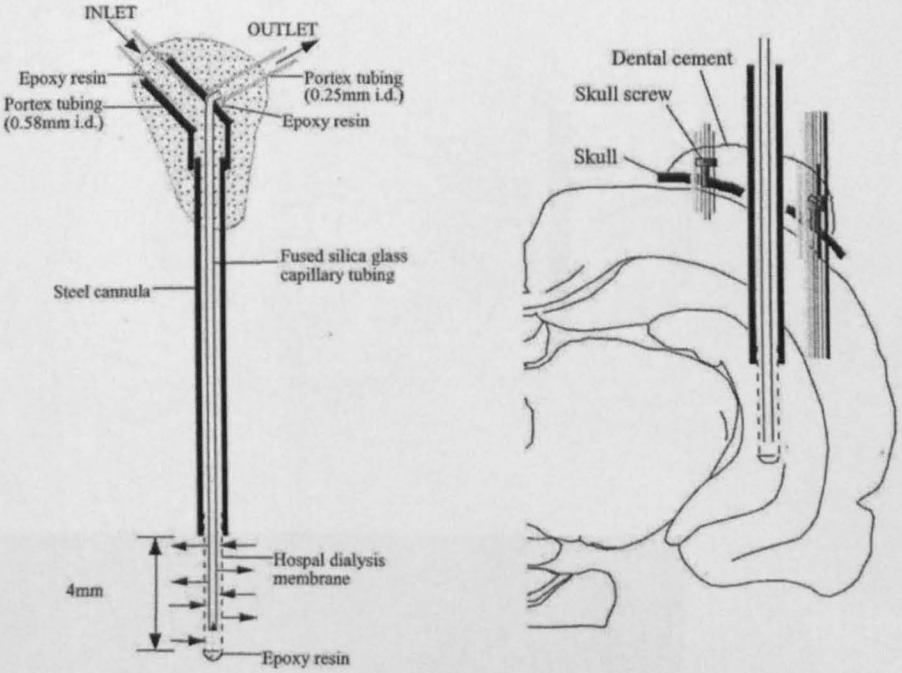
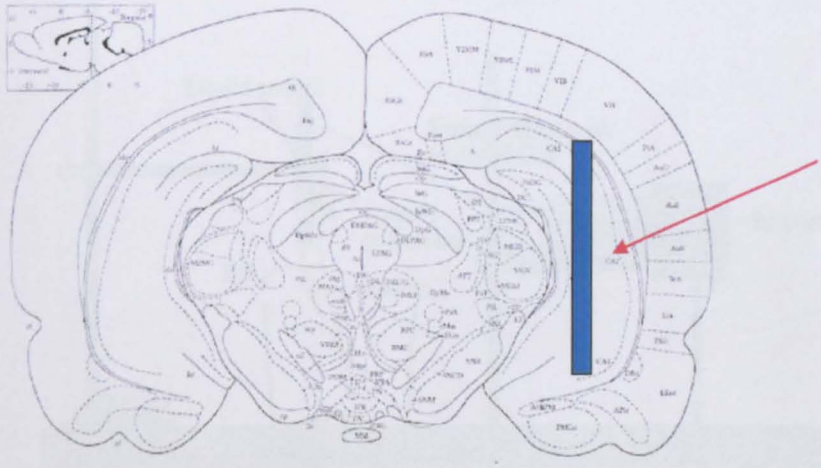


Figure 3.4 **A:** Diagram showing the basic structure of the microdialysis probe used in this experiment. A microdialysis probe consists of an inlet tube through which aCSF was continuously perfused. The outlet probe consists of a semipermeable membrane through which 5-HT passed from the brain into the probe for sample collection. The dialysis membrane was 4 mm long. **B:** The diagram shows a microdialysis probe implanted into the rat hippocampus. Three skull screws are fitted around the probe to ensure the dental cement effectively fixes to the skull so preventing the probe moving.

(A)



(B)

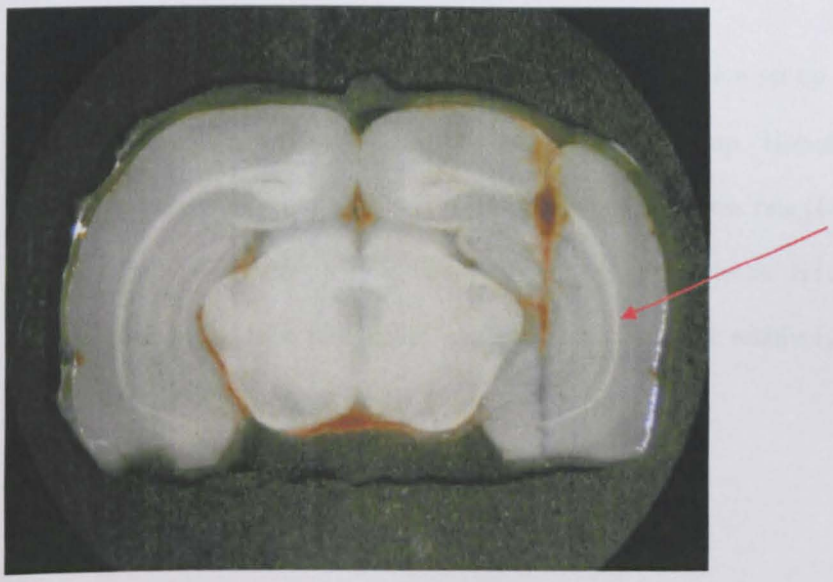


Figure 3.5 (A) shows the coordinates of the right hippocampus (AP -5.6, ML +4.6 relative to bregma and DV -8.0 below the dura) where a microdialysis probe was inserted. The picture is from Paxinos and Watson (1998). (B) Brain slice showing the staining of a microdialysis probe in the rat hippocampus. The probe placement was confirmed in all rats at the end of each experiment. Only microdialysis samples from rats in which the probe was correctly placed in the hippocampus were used for later analysis of 5-HT.

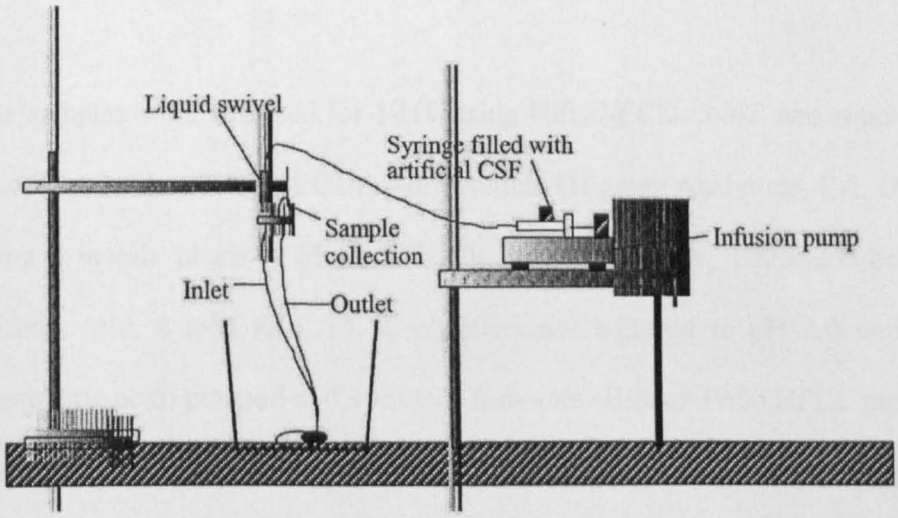
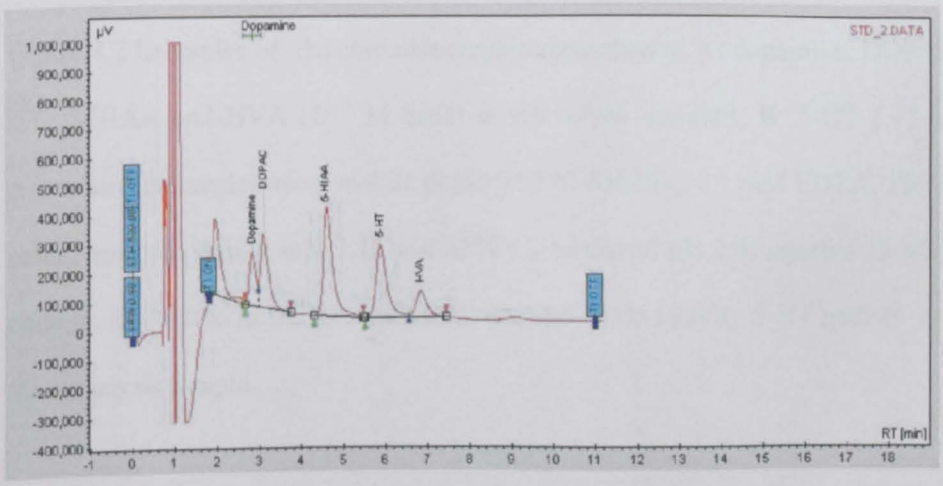


Figure 3.6 General arrangement of microdialysis probe perfusion set up. The probe is connected to a syringe pump (Harvard Microinfusion pump, Harvard Scientific, USA). The pump delivered aCSF to the probe at a constant flow rate (1.6 µl/min) via an inlet flexible connecting tube and dialysis samples were collected via an outlet. The flexible connecting tube and liquid swivel allowed the rat relatively unrestricted movement.

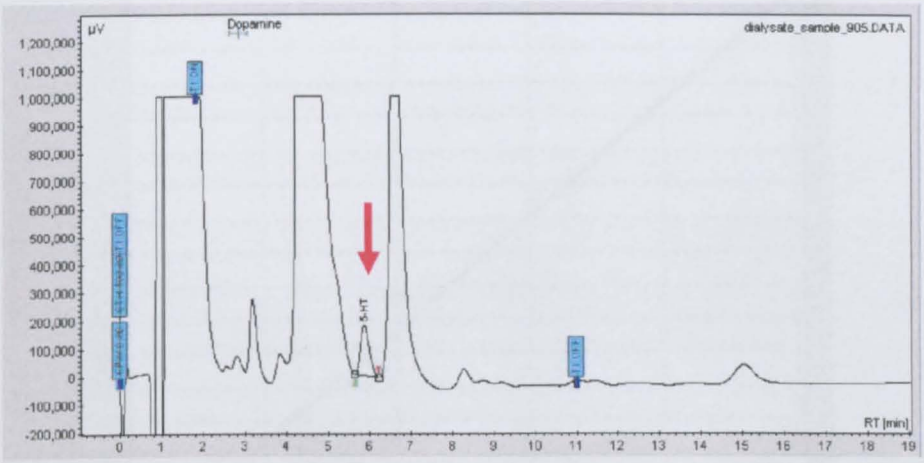
3.2.5 Measurement of 5-HT in microdialysis samples using HPLC-ECD

The samples were analysed for 5-HT using HPLC-ECD. 5-HT was separated on a 75 x 2.1 mm TARGA C18, 3-µm column (Higgins Analytical, CA, USA) using a mobile phase (0.05 M KH_2PO_4, 0.1 mM EDTA, 120 mg/L octane sulfonic acid, 8 mM KCl, 15 % v/v methanol adjusted to pH 3.0 with *o*-phosphoric acid) pumped at 0.3 ml/min flow-rate (Dionex P680 HPLC pump). Quantification was achieved using Antec VT-03 cell (Antec Leyden, Zoeterwoude, The Netherlands) with a glassy carbon working electrode set at a relative potential of +0.70 V to an *in situ* Ag/AgCl (ISAAC) reference electrode. Column and electrochemical cell were kept in a constant temperature at 32.5 °C. A 15-µl of samples and standard (10^{-8} M) were automatically injected onto the column using Series 200 Autosampler (PerkinElmer, USA). The minimum level of detection of 5-HT in this system was 7.5 fmol/15 µl sample (signal/noise ratio = 3).

(A) 10^{-8} M mixed standard of dopamine, DOPAC, HVA, 5-HT and 5-HIAA; injected 15 µl onto column



(B) An example of 5-HT in a 15 µl microdialysis sample injected onto column



(C) An overlay results of 10^{-8} M mixed standard and sample to identify the 5-HT peak in the sample (blue = standard, red = sample)

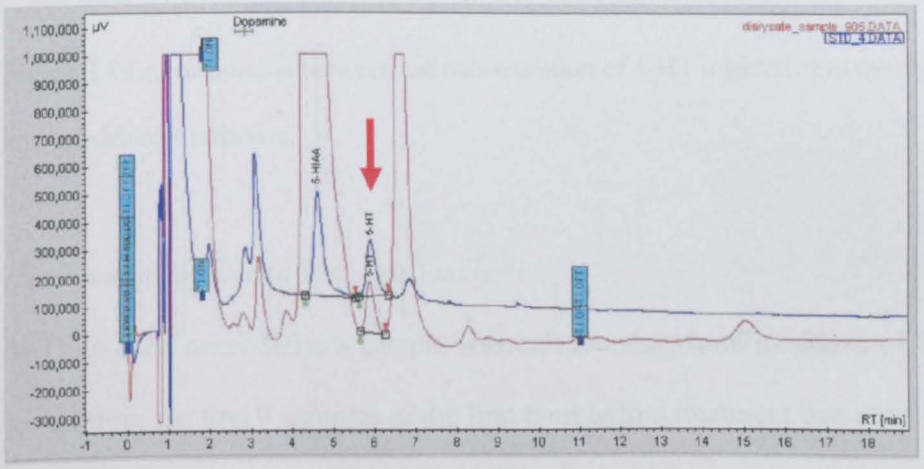


Figure 3.7 Examples of the chromatographic separation of **A:** dopamine, DOPAC, 5-HT, 5-HIAA and HVA (10^{-8} M each) in the mixed standard, **B:** 5-HT (↓) in the microdialysis samples using mobile phase 0.05 M KH_2PO_4, 0.1 mM EDTA, 120 mg/L octane sulfonic acid, 8 mM KCl and 15% v/v methanol pH 3.0; injected 15 µL onto column. C: An overlay of the result from (A) and (B) to identify 5-HT peak (↓) in the microdialysis sample.

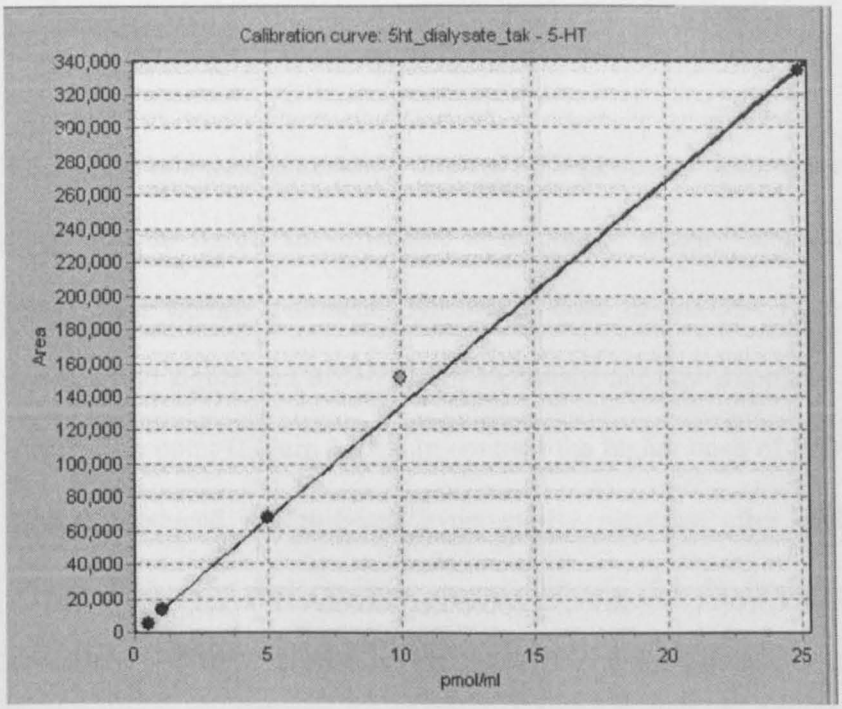


Figure 3.8 Linear relation between the concentration of 5-HT injected onto the column and the detector response.

Data manipulation and statistical analysis

5-HT in each microdialysis sample was calculated as fmol/µl. Mean of 5-HT levels from the first 3 samples in the first hour before treatment was used as an individual baseline. Data then were calculated as a percentage of an individual rat baseline (when the baseline of each rat was 100%) thereby providing mean ± SEM of each group at each time point. Two-way ANOVA with time and treatment as main factors was used to compare the change in 5-HT levels followed by Bonferroni *post hoc* test. $P < 0.05$ was considered as a significant difference.

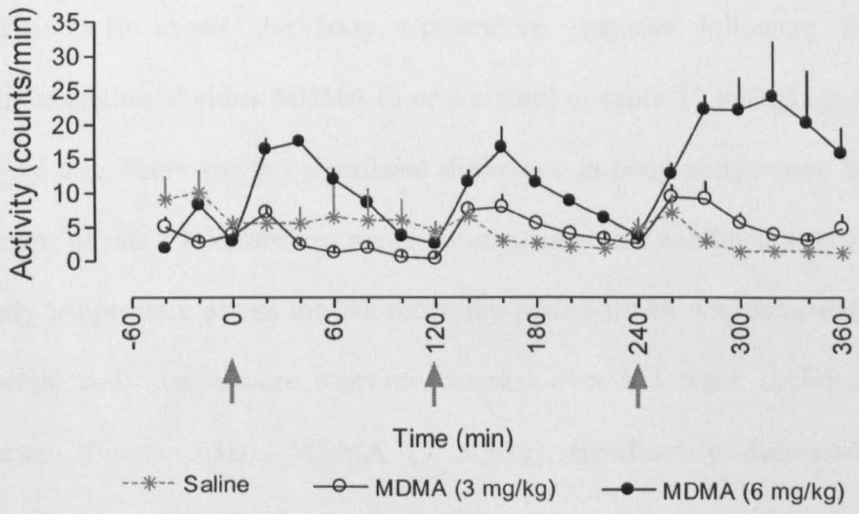
3.3. Results

3.3.1 Effect of repeated administration of MDMA on locomotor activity

There was no significant difference in locomotor activity between groups of rats 1 hour before treatment. The lower dose of MDMA (3 x 3 mg/kg) produced no significant difference in locomotor activity compared to controls at any time point (Figure 3.9A). In contrast the higher dose of MDMA (3 x 6 mg/kg) produced a significant hyperactivity response after each injection (Figure 3.9A). The peak response occurred 40 min after the first ($p < 0.05$) and second ($p < 0.01$) injection of MDMA (3 x 6 mg/kg) and a marked and sustained hyperactivity was presented following the last injection ($p < 0.001$ at t = 280- 360 compared to saline (Figure 3.9A).

Two-way ANOVA showed significant time ($F(2,28) = 3.57$, $p = 0.04$), treatment ($F(2,28) = 16.84$, $p = 0.0002$) and time x treatment interaction ($F(4,28) = 4.00$, $p = 0.011$) on the overall changes over 2 h periods after each injection (AUC) (Figure 3.9B). There was a significant lower locomotor activity response to the lower dose of MDMA (3 mg/kg) compared to the higher dose (6 mg/kg) after the first and the third injection ($p < 0.05$ and $p < 0.001$ respectively) (Figure 3.9B). In addition there was a marked response following the third injection of MDMA (6 mg/kg) ($p < 0.001$ compared to saline at the same period).

(A)



(B)

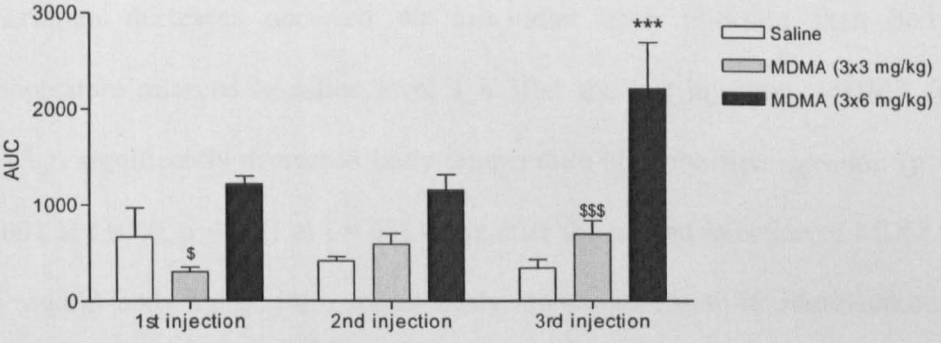


Figure 3.9 Effect of repeated administration of MDMA (3 and 6 mg/kg i.p.) and saline (1 ml/kg i.p.) 3 injections given at 2-h intervals on locomotor activity recorded using radiotelemetry. **A**: Data are presented as means of locomotor activity in 20-min time bins ± SEM (injections indicated by arrows, n=5-6 per group). There were overall effects of time ($F(26,364) = 4.00$, $p<0.0001$), treatment ($F(2,364) = 16.64$, $p = 0.0002$) and time x treatment interaction ($F(52,364) = 3.89$, $p<0.0001$) (repeated, 2-way ANOVA). The significant difference from Bonferroni *post hoc* test is not shown for the clarity of the figure. **B**: The total activity over the 2-h period after each injection (AUC). ***$p<0.001$ compared to saline; $^{\$}p<0.05$, $^{\$\$\$}p<0.001$ compared to MDMA (6 mg/kg) at the same time periods (Bonferroni *post hoc* test).

3.3.2 Effect of repeated administration of MDMA on body temperature

Figure 3.10 shows the body temperature response following repeated administration of either MDMA (3 or 6 mg/kg) or saline (1 ml/kg) i.p. 3 times every 2 h. There was no significant difference in body temperature between groups of rats 1 h before treatment. Saline treated rats exhibited a decrease of body temperature across the 7-h recording period which was comparable to a normal body temperature baseline recorded over a 1 week acclimatization period (Figure 3.3B). MDMA (3 mg/kg) significantly decreased body temperature after each injection compared to saline control ($p < 0.001$ at $t = 40$, 60, 160, 180, $p < 0.01$ at $t = 140$, $p < 0.05$ at $t = 280$) (Figure 3.10). The maximum decreases occurred 40 min after each injection then body temperature returned to saline level 1 h after the last injection. MDMA (6 mg/kg) significantly decreased body temperature after the first injection ($p < 0.001$ at $t = 40$, $p < 0.01$ at $t = 60$) while after the second injection of MDMA (6 mg/kg) body temperature continuously increased. The third administration of the higher dose of MDMA (6 mg/kg) produced a marked and sustained increase of body temperature ($p < 0.01$ at $t = 300$, $p < 0.001$ at $t = 320$-360) (Figure 3.10).

The maximum change in body temperature after each injection of either MDMA (3 or 6 mg/kg) or saline (1 ml/kg) is presented in Figure 3.11. The maximum change was calculated by subtraction of body temperature of an individual rat from the mean value 1 h before treatment then providing the mean of maximum change of each group ± SEM in each period after injection. It was shown that MDMA (3 mg/kg) and MDMA (6 mg/kg) produced a

maximum decrease of body temperature by -0.9 ± 0.1 and -0.8 ± 0.3 °C respectively following the first injection. MDMA (3 mg/kg) further decreased body temperature by -1.2 ± 0.2 and -0.4 ±0.4 °C after the second and the third injections respectively while rats treated with MDMA (6 mg/kg i.p.) continuously increased body temperature following the second injection to the maximum increase of +1.3 ± 0.5 °C after the last injection.

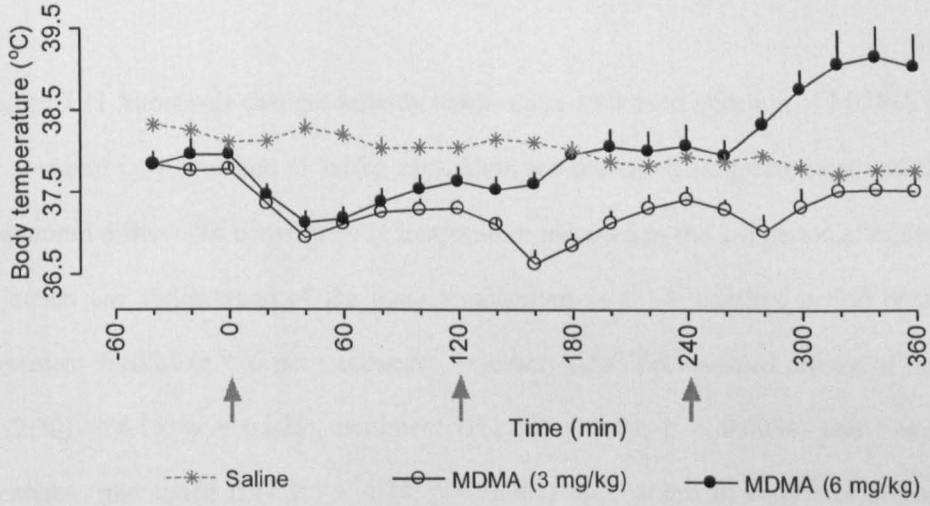


Figure 3.10 Effect of repeated administration of either MDMA (3 or 6 mg/kg i.p.) or saline (1 ml/kg i.p.), 3 injections given at 2-h intervals, on body temperature recorded using radiotelemetry. Data are presented as means of body temperature in 20-min time bins ± SEM (injections indicated by arrows, n = 6 per group). Two-way ANOVA showed significant effects of time ($F(26,390)=5.67$, $p<0.0001$), treatment ($F(2,390)=20.16$, $p<0.0001$) and time x treatment interaction effects ($F(52,390)=7.91$, $p<0.0001$). The significant difference from Bonferroni *post hoc* test is not shown for the clarity of the figure.

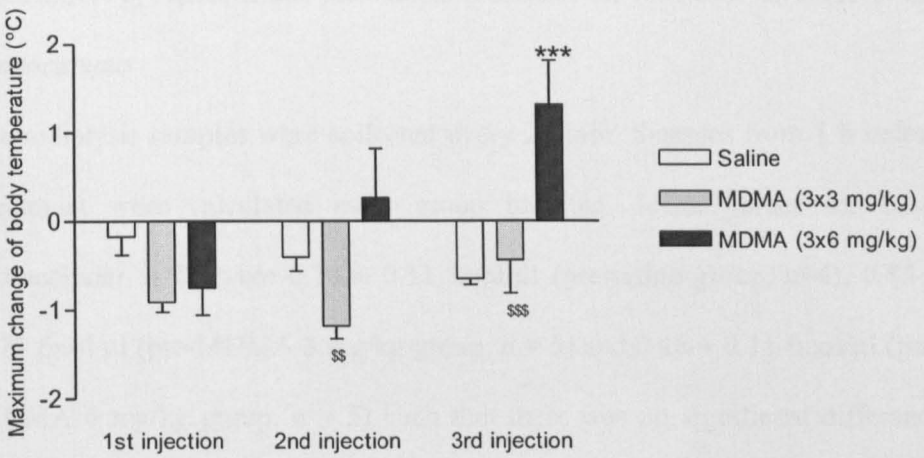


Figure 3.11 Maximum changes in body temperature after each injection of MDMA (3 or 6 mg/kg i.p.) or saline (1 ml/kg i.p.). Data are presented as group means of the maximum differences between body temperature recorded in the 2-h period after each injection and the average of the body temperature in a 1-h baseline period before treatment ± SEM (n = 6 per treatment). Two-way ANOVA revealed effects of time ($F(2,30) = 4.15$, $p = 0.026$), treatment ($F(2,30) = 8.53$, $p = 0.0034$) and time x treatment interaction ($F(4,30) = 4.74$, $p = 0.004$) on changes in body temperature. ***$p<0.001$ compared to saline control at the same period, $^{\$\$}p<0.01$, $^{\$\$\$}p<0.001$ compared to MDMA (6 mg/kg) at the same time period (Bonferroni *post hoc* test)

3.3.3 Effect of repeated administration of MDMA on extracellular 5-HT in the hippocampus

Microdialysis samples were collected every 20 min. Samples from 1 h before treatment were calculated as a group baseline. Mean values for basal extracellular 5-HT were 0.73 ± 0.11 fmol/µl (pre-saline group, n=4), 0.84 ± 0.22 fmol/µl (pre-MDMA 3 mg/kg group, n = 5) and 0.48 ± 0.11 fmol/µl (pre-MDMA 6 mg/kg group, n = 5) such that there was no significant difference between groups of rats ($F_{2,13} = 1.334$, $p = 0.303$; one-way ANOVA).

Figure 3.12 shows effect of repeated administration of MDMA (3 and 6 mg/kg) and saline (1 ml/kg) on extracellular levels of 5-HT in the hippocampus. Data are presented as means of % baseline calculated compared to means of the first three samples (see 3.2.4). Two-way ANOVA revealed time ($F(21,225) = 1.87$, $p = 0.0139$) and treatment effects ($F(2,225) = 39.75$, $p < 0.0001$) on extracellular 5-HT levels in the hippocampus (Figure 3.12). The higher dose of MDMA (6 mg/kg i.p.) produced a marked increase in extraneuronal 5-HT levels in the hippocampus 40, 60 and 80 min after the first injection (+485, +555 and +393% respectively compared to the 100% basal levels) while the lower dose of MDMA (3 mg/kg) increased 5-HT to a peak increase of +134% compared to 100 % baseline 40 min after the first injection. After the second injection, MDMA (6 mg/kg) produced a maximum increase of +389% compared to 100% baseline at t = 180 while MDMA (3 mg/kg) increased 5-HT by +284 % compared to 100% baseline at t = 180. After the third injection, the magnitude of increase of 5-HT following both doses of MDMA was similar as

the maximum increase of + 315 % and + 292 % compared to 100 % baseline by MDMA 6 and 3mg/kg respectively at t = 300 (Figure 3.12).

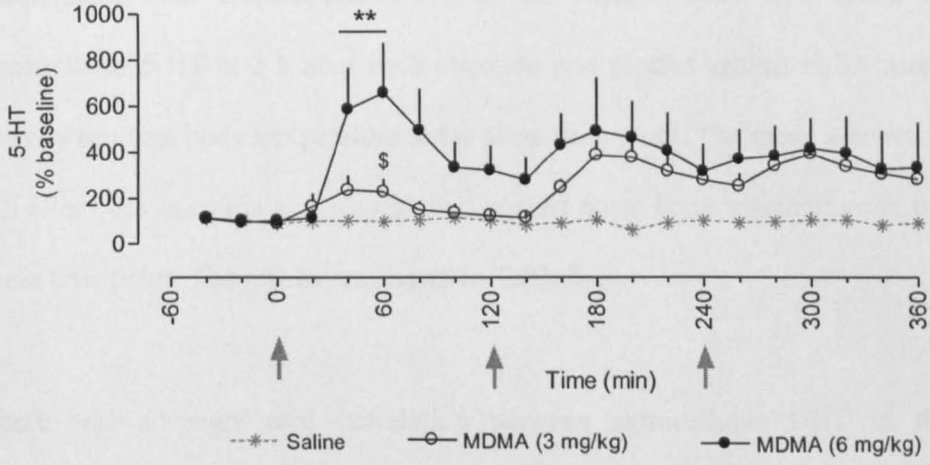


Figure 3.12 Effect of repeated administration of MDMA on extracellular 5-HT levels in the hippocampus. Each point represents % baseline calculated compared to means of the first three samples. Two-way ANOVA revealed time ($F(21,226) = 1.86$, $p = 0.0127$) and treatment effects ($F(2,226) = 39.88$, $p < 0.0001$) on extracellular 5-HT levels in the hippocampus. $^{**}p < 0.01$ compared to saline at the same time point, $^{\$}p < 0.05$ compared to MDMA (3 x 6 mg/kg) at the same time point (Bonferroni *post hoc* test)

3.3.4 Correlation

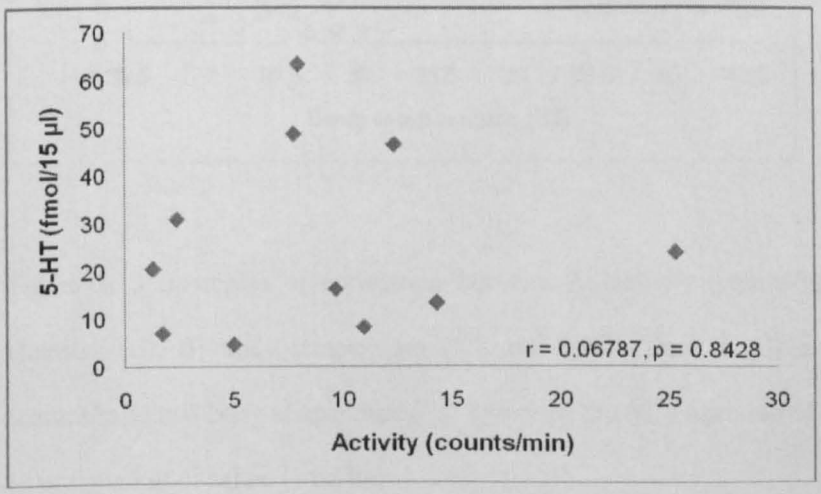
Pearson correlation analysis was applied to determine whether there was a relation between the functional changes (locomotor activity and body temperature) and extracellular 5-HT in the hippocampus. The mean of extracellular 5-HT at 2 h after each injection was plotted against either mean activity or mean body temperature at the same time point. The mean activity in 2 h after each injection was also plotted against mean body temperature at the same time point. The results are shown in Table 3.1.

There was no significant correlation between extracellular 5-HT in the hippocampus and either body temperature or locomotor activity after each injection (Table 3.1). There was no significant correlation between body temperature and locomotor activity after the first and second injections. However there was a significant positive correlation between body temperature and locomotor activity (Pearson correlation coefficient; $r = 0.8072$; $p < 0.0001$) (Table 3.1).

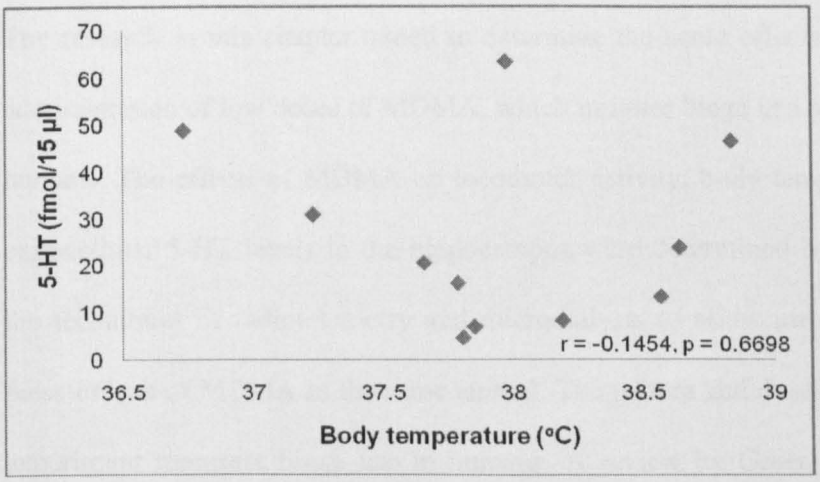
Table 3.1 Data showing lack of correlations between extracellular 5-HT in the hippocampus and either locomotor activity or body temperature while there was a correlation between locomotor activity and body temperature but only after the third injection of MDMA. Data were calculated from mean of extracellular levels of 5-HT in the hippocampus, mean activity and mean body temperature in 2 h period after each injection. Data are presented in Pearson correlation coefficients (r) with corresponding p value.

Correlation	1st injection			2nd injection			3rd injection		
	r	p	Sig	r	p	Sig	r	p	Sig
Activity vs 5-HT	0.1337	0.6951	ns	0.1420	0.6771	ns	0.06787	0.8428	ns
Body temp vs 5-HT	-0.5696	0.0674	ns	-0.3936	0.2310	ns	-0.1454	0.6698	ns
Activity vs Body temp	0.3746	0.1256	ns	0.3634	0.1383	ns	0.8072	< 0.0001	***

(A) Correlation between activity (counts/min) and 5-HT (fmol/15 µl) after the third injection



(B) Correlation between body temperature (°C) and 5-HT (fmol/15 µl) after the third injection



(C) Correlation between activity (counts/min) and body temperature (°C) after the third injection

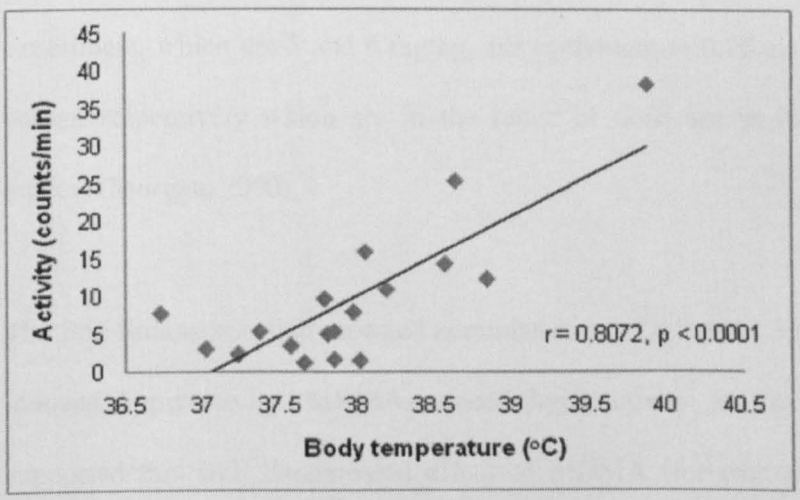


Figure 3.13 Examples of correlation between **A:** activity (counts/min) and 5-HT (fmol/15 µl), **B:** body temperature (°C) and 5-HT (fmol/15 µl) and **C:** activity (counts/min) and body temperature (°C) following the third injection of either MDMA (3 or 6 mg/kg) or saline (1 ml/kg)

3.4. Discussion

The research in this chapter aimed to determine the acute effects of repeated administration of low doses of MDMA, which imitates binge use of the drug in humans. The effects of MDMA on locomotor activity, body temperature and extracellular 5-HT levels in the hippocampus were determined by combining the techniques of radiotelemetry and microdialysis to allow measurement of these effects of MDMA in the same animal. The pattern and doses used in this experiment represent binge use in humans. A review by Green et al (2009) comparing MDMA dose-plasma concentration response in rats and human has suggested that "a fourfold higher dose is required in rats to produce a similar peak plasma MDMA concentration in humans". Therefore doses used in this experiment, which are 3 and 6 mg/kg, are equivalent to 0.75 and 1.5 mg/kg in human respectively which are in the range of dose use in humans (0.75-4 mg/kg) (Morgan, 2000).

The first finding was that repeated administration of 6 but not 3 mg/kg MDMA induced hyperactivity. MDMA-induced hyperactivity in the present study supported this well documented effect of MDMA (for example Kindlundh-Hogberg et al., 2007, Spanos and Yamamoto, 1989). Additionally the present study provided evidence of marked and sustained hyperlocomotor activity when MDMA was given repeatedly. In addition to the acute effect on locomotor activity, it was demonstrated in the present study that both doses of MDMA produced different body temperature responses. The lower dose of MDMA (3 mg/kg) decreased body temperature following each injection while

the higher dose of MDMA (6 mg/kg) produced hypothermia after the first injection then continuously increased body temperature after the second injection to the peak increase of +1.3 °C after the last injection.

MDMA-induced change in body temperature depends mainly on an ambient room temperature (Dafters and Lynch, 1998, Green et al., 2004, Malberg and Seiden, 1998). The ambient temperature in this experiment was set at 21±1 °C and the body temperature response to MDMA reported at this ambient temperature range is controversial as some studies reported MDMA-induced hyperthermia (Dafters and Lynch, 1998, Mechan et al., 2002a) while others found hypothermia (Jaehne et al., 2005, Malberg and Seiden, 1998). It is noticeable that all of these previous experiments used a single high dose of MDMA (10 - 40 mg/kg) while in the present study, using repeated low doses (3 or 6 mg/kg), there was hypothermia after the first administration.

Following repeated injection, the lower dose of MDMA (3 mg/kg) decreased body temperature after the second and third injections. This result is in contrast to the study by Green et al (2004) in which they reported a dose-dependent hyperthermia in rats housed at 19 °C following repeated administration of low doses of MDMA (2, 4 and 6 mg/kg, 3 injections every 3 h). It is noteworthy that this study by Green et al (2004) was conducted in group housed rats whereas rats in the present study were singly housed as this is required for the radiotelemetry measurement. Housing condition is a factor that influence MDMA-induced changes in body temperature. It has been demonstrated that amphetamines including MDMA produced more marked behavioural effects in

group than single housed animals an effect called 'aggregate toxicity' (Chance, 1947, Fantegrossi et al., 2003, Morton et al., 2001). Group housing of the rats also well represents a crowded dance club condition where MDMA is normally used in humans. Therefore not only ambient temperature but also the single housing condition in the present study possibly influenced the hypothermia caused by the lower dose of MDMA (3 mg/kg).

In contrast to the lower dose of MDMA, changes in thermoregulation were found after repeated administration of the higher dose of MDMA (6 mg/kg). This effect of repeated higher dose MDMA might involve the impairment of central thermoregulation in the hypothalamus as well as other heat loss mechanisms. It has been previously shown that MDMA inhibited the rat tail vasodilatory heat loss mechanism (Blessing et al., 2003, Green et al., 2005) and also affected other physiological mechanisms associated with thermoregulation (Gordon et al., 1991).

It has been suggested previously that changes in body temperature observed after MDMA administration might result from MDMA-induced hyperactivity; however, some studies have shown dissociation between MDMA-induced hyperthermia and hyperlocomotion (Dafters, 1994, 1995). The present study found no correlation of changes in body temperature and locomotor activity following the first and second injections of MDMA while there was significant positive correlation between changes in body temperature and locomotor activity after the third injection. This result indicates that MDMA-induced hyperthermia is partly influenced by an increase of locomotor activity.

MDMA administration to rats has been shown to induce an acute and rapid release of 5-HT in various brain regions including striatum, prefrontal cortex, nucleus accumbens and hippocampus (Baumann et al., 2008b, Mechan et al., 2002a, Stanley et al., 2007). Mechan et al (2002a) showed an increase of 5-HT in the hippocampus following a single dose of MDMA (15 mg/kg). This is in agreement with the present study which demonstrated an increase of extracellular 5-HT in the hippocampus using low doses of MDMA (3 and 6 mg/kg). The higher dose of MDMA (6 mg/kg) produced a greater release of 5-HT in the hippocampus after the first injection compared to the lower dose of MDMA (3 mg/kg) but then the magnitude of 5-HT release was similar after the second and third administration. The lesser release of 5-HT following repeated administration of the higher dose of MDMA might be due to an inhibition of tryptophan hydroxylase activity in various brain regions including the hippocampus (Che et al., 1995, Johnson et al., 1992, O'Shea et al., 2006, Stone et al., 1987b) which occurs in the rat 15 min after MDMA administration (Stone et al., 1987b) together with depletion of 5-HT within synaptic vesicles as MDMA (6 mg/kg) produced a greater release of 5-HT after the first injection. Inhibition of 5-HT synthesis and depletion of 5-HT storage caused by the higher dose of MDMA (6 mg/kg) may account for the consequent decrease in the magnitude of 5-HT release after repeated dosing.

There was no correlation between extracellular 5-HT in the hippocampus and locomotor activity in this study. MDMA-induced hyperactivity has been suggested to be involved in a complex interaction of 5-HT and dopamine release following MDMA administration (Bankson and Cunningham, 2001).

Recently Baumann et al (2008) showed a direct correlation between locomotor activity with dialysate 5-HT and dopamine with a positive correlation between ambulation and dialysate dopamine levels in the nucleus accumbens, striatum and prefrontal cortex and with dialysate 5-HT levels in the striatum and prefrontal cortex. Therefore the determination of extracellular 5-HT levels in the hippocampus in the present study indicates that hippocampal 5-HT release is not involved in MDMA-induced locomotor activity.

There was also no correlation between extracellular 5-HT in the hippocampus and body temperature. The mechanism underlying MDMA-induced hyperthermia remains unclear however 5-HT and dopamine systems have been suggested to mediate this effect of MDMA as the 5-HT_{2A} antagonists, ritanserin, ketanserin, M100907 and R-96544 as well as the dopamine D_1 antagonist, SCH 23390 can protect against MDMA-induced hyperthermia (Benamar et al., 2008, Herin et al., 2005, Mechan et al., 2002a, Shioda et al., 2008). MDMA-induced 5-HT release in the hypothalamus might mediate this effect of MDMA as 5-HT is suggested to mediate thermoregulatory function in the hypothalamus (see review Bligh, 1979). Therefore it is unlikely that the hippocampus is the locus of any 5-HT mediated thermoregulatory response.

In summary, the research in this chapter provides evidence for acute effects of repeated administration of low doses of MDMA. The present study shows that repeated administration of MDMA increases the risk of thermoregulatory impairment especially when using the higher dose (6 mg/kg). In addition the present study also demonstrated an increase of extracellular 5-HT in the

hippocampus following low doses of MDMA. Although this increase in 5-HT released failed to correlate with locomotor activity and body temperature, the increase of extracellular 5-HT in the hippocampus, the brain region related to learning and memory (Mumby, 2001), might explain the impairment of novel objection discrimination following acute MDMA (3 mg/kg) administration demonstrated in the previous chapter. In addition to the acute effects, long-term clinical complications including impairments of learning and memory have been widely reported especially in frequent and heavy MDMA users (see review Gouzoulis-Mayfrank et al., 2000). Therefore the consequence of acute 5-HT release in the hippocampus and change in body temperature following repeated administration of MDMA was further investigated in the following chapter in terms of its effects on 5-HT neurotoxicity and impairment of learning and memory.

CHAPTER 4

THE LONG-TERM EFFECTS OF REPEATED MDMA ADMINISTRATION ON MEMORY AND 5-HT NEUROTOXICITY

4.1. Experiment 1: Acute effects of repeated MDMA administration to singly housed rats on locomotion and body temperature and the long-term effects on novel object discrimination and neurotoxicity

4.1.1 Introduction

In the previous chapter it was demonstrated that repeated administration of low doses of MDMA (3 and 6 mg/kg 3 times every 2 h) increased extracellular 5-HT in the hippocampus. The first experiment in this chapter consequently investigated the long-term effects of repeated MDMA administration on recognition memory and 5-HT neurotoxicity.

In adult rats, MDMA administration has been shown to produce long-term cognitive deficits using various cognition models. For example, MDMA treatment produced long-term spatial memory deficits in the Morris water maze (MWM) (Able et al., 2006, Sprague et al., 2003). Able et al (2006) and Skelton et al (2008) also reported an impairment of path integration learning in the Cincinnati water maze (CWM) following repeated MDMA treatment in rats. In addition MDMA-treated rats showed deficiencies in a test of novel object recognition (McGregor et al., 2003, Morley et al., 2001).

MDMA-induced long-term 5-HT depletion has been reported in rats especially after high dosage regimens (see review Capela et al., 2009, Green et al., 2003). The relevance of the 5-HT neurotoxicity to the memory impairments remains unclear. Sprague et al (2003) showed that MDMA (2x20 mg/kg) treatment resulted in decreased hippocampal 5-HT 2 weeks after treatment together with

an impairment of spatial memory using the MWM. McGregor et al (2003) found an impairment of novel object recognition and a decrease 5-HT levels in the hippocampus, amygdala, striatum and cortex. Able et al (2005) and Skelton et al (2008) reported a decrease of 5-HT in hippocampus, neostriatum and prefrontal cortex associated with an impairment of path integration learning in the CWM but no change in novel object recognition performance. Overall these results indicate a possible link between 5-HT loss and memory impairment.

The aim of the first experiment in this chapter was to investigate the long-term effect of repeated administration of low doses MDMA on memory using novel object discrimination. Rats were individually housed in the activity monitor chamber during MDMA treatment to imitate the singly housing conditions described in the previous chapter. Locomotor activity, using a non telemetric system, and rectal temperature were also monitored during MDMA administration. In addition changes in 5-HT, dopamine and their metabolites were determined 2 weeks after MDMA administration in specific brain regions considered to be involved in cognitive processes.

4.1.2 Materials and methods

Animals

Adult male Lister hooded rats (Charles River, UK) weighing 300-350 g were housed on a 12 h light/dark cycle (light on at 07.00h) in constant room temperature (21±2 °C) and humidity (45-65%). Rats had free access to food and water. All experiments were performed in accordance with the UK Animals (Scientific Procedures) Act, 1986 under project license 40/2715 and approved by the University of Nottingham Local Ethical Review Committee.

Experimental procedure

Rats were housed in groups of 4 for 1 week before treatment. On the day before treatment, each rat was habituated in an individually computer-controlled infra-red activity monitor chamber (a 39 x 23 cm with 30 cm high clear Perspex box) for 1 h (Figure 4.2). The locomotor activity was recorded during this period to determine whether each group of rats showed a level of normal activity before treatment. On the treatment day each rat was singly placed in an individual infra-red activity monitor chamber during treatment in order to measure the acute effects of repeated low doses of MDMA on locomotor activity. Rats had free access to water during this period. Rats were given either MDMA (3 mg/kg or 6 mg/kg i.p.) or saline (1 ml/kg i.p.) 3 times every 2 h (n = 8 per treatment). MDMA was synthesized by Department of Chemistry, University College Dublin, Ireland. Activity was recorded from 1 h before treatment until 2 h after the last injection. Before the rats were returned to their own home cages rectal temperature was measured using a digital thermocouple probe thermometer (Portec Instrumentation Ltd, P9005) inserted

6-8 cm into the rectum using light restraint. On day 14 each rat was habituated in an individual infra-red activity monitor chamber for 1 h and locomotor activity recorded. On the following day the novel object discrimination task was performed as described in chapter 2 with an inter-trial interval of 2 h. Rats were killed immediately after the novel object discrimination task. Three brain regions; frontal cortex, hippocampus and striatum, were dissected and later analysed for 5-HT, dopamine and their metabolites using HPLC-ECD as described in chapter 2. (Experimental design summarised in Figure 4.1)

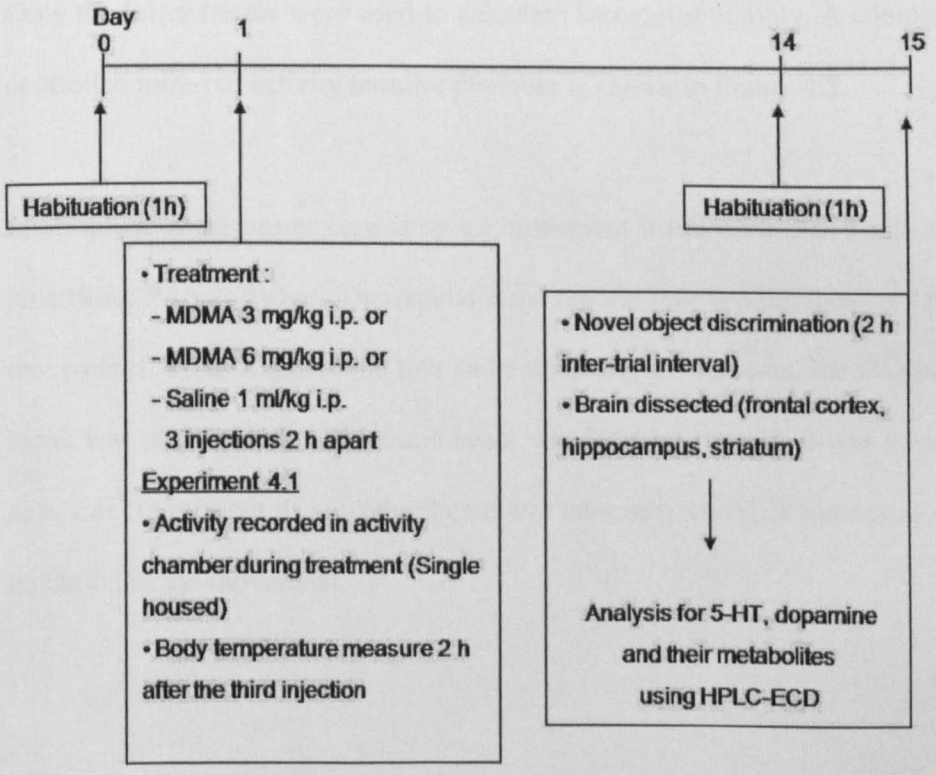


Figure 4.1 Experimental procedure to determine the effects of repeated administration of low doses of MDMA to singly housed rats on novel object discrimination, and brain region 5-HT, dopamine and their metabolites levels.

Locomotor activity

To record activity each rat was placed in a computer-controlled infra-red activity monitor chamber (SDI's photobeam activity system, San Diego Instruments, San Diego, USA) which consisted of a clear Perspex box (39 x 23 cm with 30 cm high), with solid Perspex floor and a wire mesh lid. Four parallel infra-red beams, 4-cm apart, cross the chamber referred as X-beams and 8 parallel infra-red beams, 5-cm apart, cross the chamber referred as Y-beams at 3.5-cm above floor level recorded locomotion. The upper layer beam, set at 15.5-cm above floor levels with 8 beams 2.5-cm apart, recorded rearing. Only the lower beams were used to calculate locomotor activity. A computer-controlled infra-red activity monitor chamber is shown in Figure 4.2.

Interruption of the beams caused by rat movement was recorded in 5-min time bins using PAS software. The results were reported as 'ambulatory' or 'fine' movements. To distinguish the fine and ambulatory movements, the first beam break was not counted. If the same beam was later interrupted, it was counted as a 'fine' movement. If any other beam was later interrupted, it was counted as an 'ambulatory' movement.

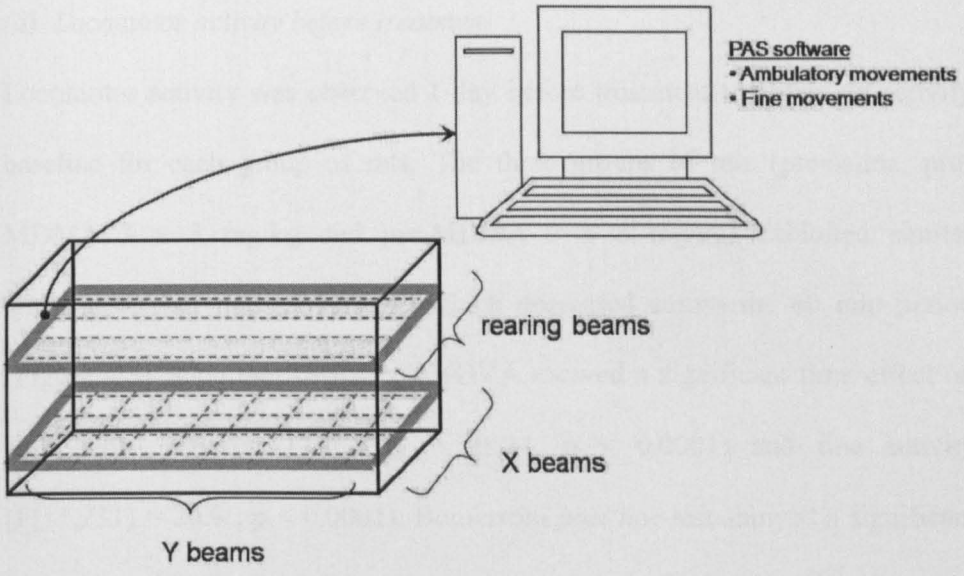


Figure 4.2 A computer-controlled infra-red activity monitor chamber (SDI's photobeam activity system, San Diego Instruments, San Diego, USA). The system consisted of a clear Perspex box (39 x 23 cm with 30 cm high), 4-parallel infra-red X beam and 8-parallel infra-red Y beam at 3.5-cm above floor level recorded locomotions and 4-infra-red rearing beam 15.5-cm above floor level recorded rearing and PAS software intregrated and reported the results as 'ambulatory' movement, 'fine' movement and rearing.

Statistical analysis

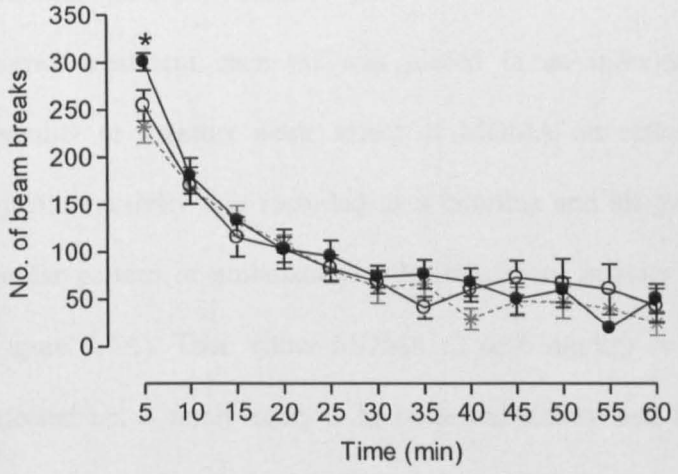
Two-way ANOVA followed by Bonferroni *post hoc* test, one-way ANOVA followed by Tukey's *post hoc* test, unpaired Student *t*-test and one sample *t*-test were used where appropriate. $P < 0.05$ was considered as a significant difference.

4.1.3 Results

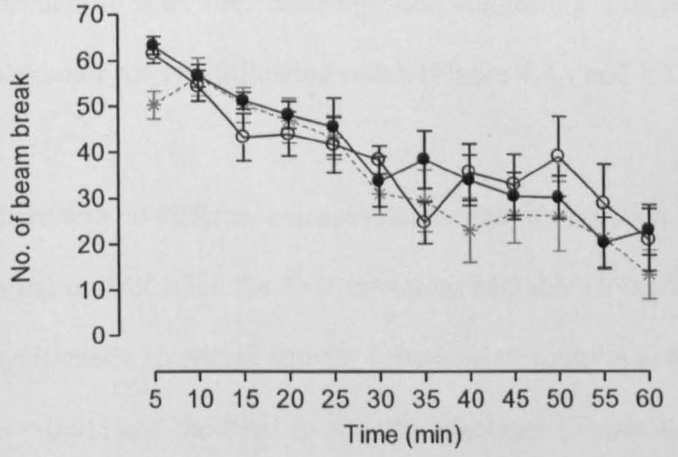
(a) Locomotor activity before treatment

Locomotor activity was observed 1 day before treatment to obtain an activity baseline for each group of rats. The three groups of rats (pre-saline, pre-MDMA 3 x 3 mg/kg and pre-MDMA 3 x 6 mg/kg) exhibited similar ambulatory and fine movements which decreased across the 60 min period (Figure 4.3), such that two-way ANOVA showed a significant time effect on ambulatory activity ($F(11,231) = 91.24$, $p < 0.0001$) and fine activity ($F(11,231) = 20.9$, $p < 0.0001$). Bonferroni *post hoc* test showed a significant difference in the ambulatory activity between pre-saline and pre-MDMA (3 x 6 mg/kg) treated group in the first 5 min ($p < 0.05$) but there was no significant difference in either ambulatory or fine movement at any other time points. Thus although the pre-MDMA (3 x 6 mg/kg) were more active than the other 2 groups at the beginning, overall all 3 groups of rats showed a similar pattern of activity before treatment (Figure 4.3).

(A)



(B)



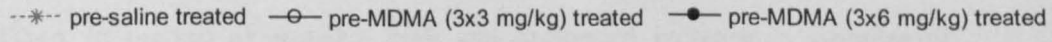


Figure 4.3 **A:** Ambulatory and **B:** fine activity 1 day before treatment. Each point represents mean of number of beam breaks in 5-min ± SEM (n = 8 per group). * $p < 0.05$ compared with saline in the same time point (Bonferroni *post hoc* test following two-way ANOVA).

(b) The acute effects of repeated administration of low doses of MDMA on ambulatory and fine activity

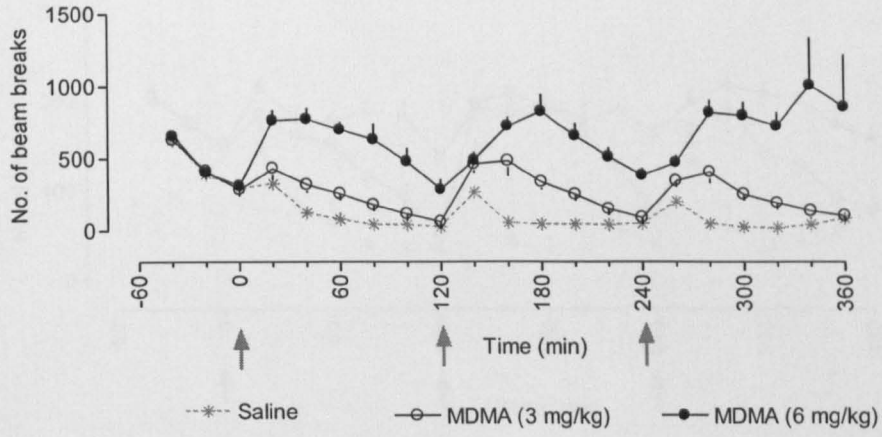
During treatment each rat was placed in an individual infra-red activity chamber to measure acute effect of MDMA on activity. One hour before injection activity was recorded as a baseline and all group of rats showed a similar pattern of ambulation with ambulatory activity decreasing over time (Figure 4.4A). Then either MDMA (3 or 6 mg/kg) or saline (1 ml/kg) was injected i.p. 3 times every 2 h. Both ambulatory and fine activity increased immediately after each injection of either MDMA (3 or 6 mg/kg) or saline (1 ml/kg), however, the activity of the rats treated with saline rapidly returned to baseline 40 min after each injection suggesting stressed-induced increase of locomotor activity following saline (Figure 4.4A and 4.5A).

There was no difference in ambulatory activity between MDMA (3 mg/kg) and saline control after the first injection. MDMA (3 mg/kg) treatment however significantly increased activity compared to controls at 40 min after the second ($p < 0.01$) and the third ($p < 0.05$) injections (Figure 4.4A). Rats treated with MDMA (6 mg/kg) exhibited a high and prolonged increase in ambulatory activity after each injection ($p < 0.01$ at $t = 20, 100, 220$, $p < 0.001$ at $t = 40\text{-}80, 160\text{-}200, 280\text{-}360$) (Figure 4.4A). Total activity showed a highly significant difference in ambulatory activity after each injection of MDMA (6 mg/kg) compared to both saline and MDMA (3 mg/kg) (Figure 4.4B).

Interestingly although the lower dose of MDMA (3 mg/kg) produced little significant difference in ambulatory activity, this dose of MDMA produced a

marked increase in fine movements after three injections of the drug ($p < 0.05$ at $t = 220$, $p < 0.01$ at $t = 40, 80, 320$, $p < 0.001$ at $t = 60, 160, 180, 200, 280, 300$) (Figure 4.5A). Total level of fine activity after all three injections of MDMA (3 mg/kg) was significantly increased compared to saline control ($p < 0.001$) (Figure 4.5B). The higher dose of MDMA (6 mg/kg) produced an increase in fine activity which was prolonged after each injection compared to saline-treated controls ($p < 0.001$) (Figure 4.5A).

(A)



(B)

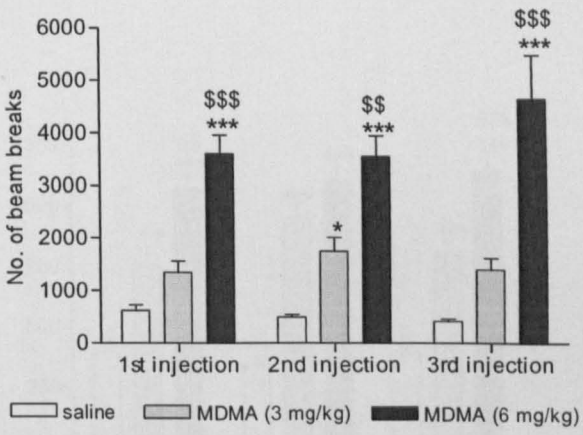
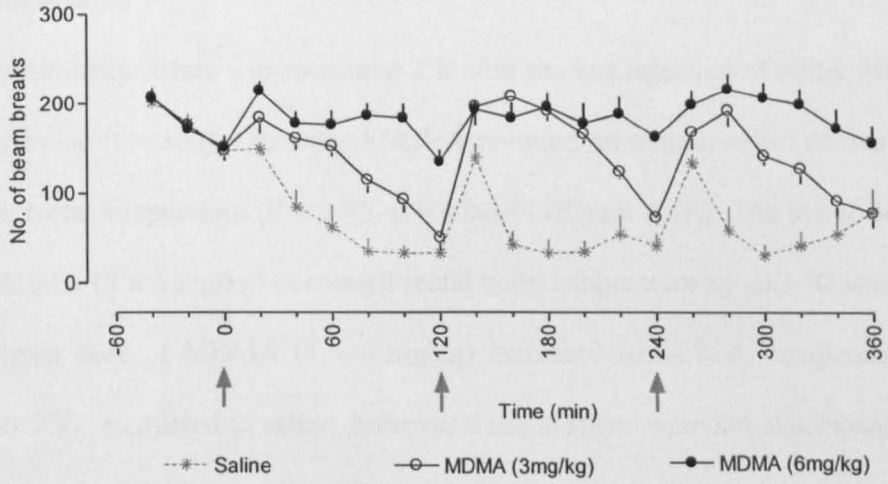


Figure 4.4 **A:** Ambulatory activity during treatment in the infra-red activity chambers. Rats were given either MDMA (3 or 6 mg/kg) or saline (1 mg/kg) i.p. 3 times every 2 h as indicated by arrows. Each point represents mean of number of beam breaks in 20-min time bins ± SEM (n = 8 per treatment). Two-way ANOVA revealed effects of time ($F(20,420) = 6.99$, $p < 0.0001$), treatment ($F(2,420) = 47.90$, $p < 0.0001$) and time x treatment interaction ($F(40,420) = 4.39$, $p < 0.0001$) on ambulatory activity. The significant difference from Bonferroni *post hoc* test is not shown for the clarity of the figure. **B:** Total ambulatory activity in 2-h time bins after each injection. * $p < 0.05$, ***$p < 0.001$ compared to saline at the same time period; $^{\$\$}$ $p < 0.01$, $^{\$\$\$}p < 0.0001$ compared to MDMA (3 mg/kg)-treated at the same time period (Bonferroni *post hoc* test following 2-way ANOVA).

(A)



(B)

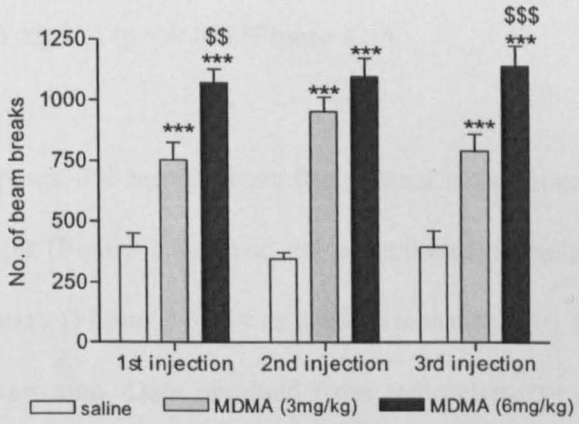


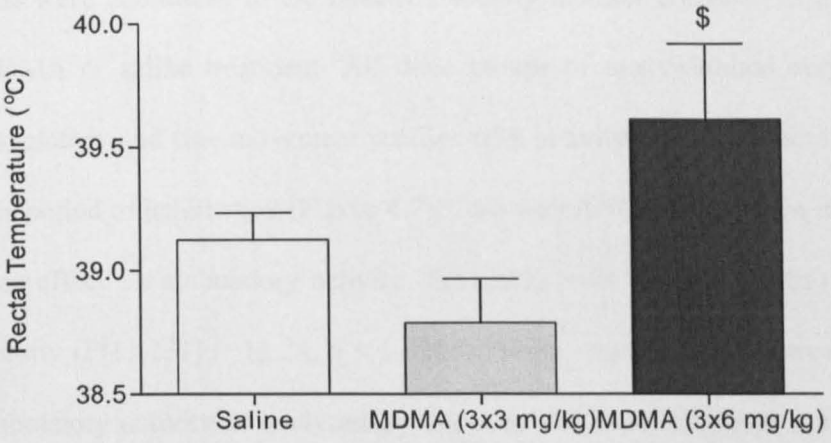
Figure 4.5 **A:** Fine activity during treatment in the infra-red activity chambers. Each point represents mean of number of beam breaks in 20-min time bins ± SEM (n = 8 per treatment). Injections were indicated by arrows. Two-way ANOVA revealed effects of time ($F(20,420) = 16.82$, $p < 0.0001$), treatment ($F(2,420) = 67.90$, $p < 0.0001$) and time x treatment interaction ($F(40,420) = 6.13$, $p < 0.0001$) on fine activity. The significant difference from Bonferroni *post hoc* test is not shown for the clarity of the figure. **B:** Total fine activity in 2-h time bins after each injection. ***$p < 0.001$ compared to saline at the same time period; $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$ compared to MDMA (3 mg/kg)-treated at the same time period (Bonferroni *post hoc* test following 2-way ANOVA).

(c) The effect of repeated administration of low doses of MDMA on rectal temperature

Rectal temperature was measured 2 h after the last injection of either MDMA or saline (t = 360). One-way ANOVA revealed an overall effect of treatment on rectal temperature ($F = 3.75$, $p = 0.0406$) (Figure 4.6A). The lower dose of MDMA (3 x 3 mg/kg) decreased rectal body temperature by -0.3 °C while the higher dose of MDMA (3 x 6 mg/kg) increased rectal body temperature by +0.5 °C compared to saline, however these changes were not significant from saline control (Figure 4.6A). Three injections of the lower dose of MDMA (3 x 3 mg/kg) significantly reduced rectal temperature compared to the higher dose of MDMA (3 x 6 mg/kg) ($p < 0.05$) (Figure 4.6A)

Figure 4.6 compares the results from the present experiment using a rectal probe thermometer (Figure 4.6A) and the experiment in the previous chapter when radiotelemetry (Figure 4.6B) was used to measure body temperature at 2 h after the last injection. Data obtained from radiotelemetry showed that the higher dose of MDMA (3 x 6 mg/kg) induced hyperthermia (+1.7 °C) and this is significantly higher than saline ($p < 0.05$). The lower dose of MDMA (3 x 3 mg/kg) decreased telemetric body temperature (-0.2 °C) compared to control at 2 h after the last injection (t = 360) and this is significantly lower than MDMA (3 x 6 mg/kg) treated group ($p < 0.01$) (Figure 4.6B).

(A)



(B)

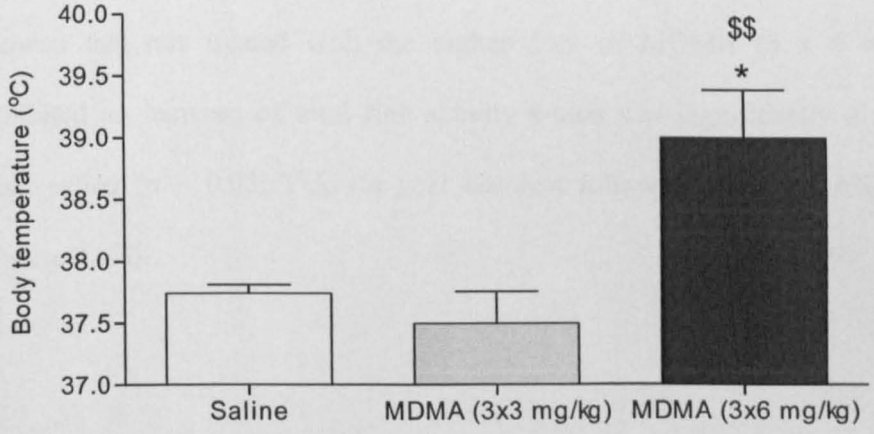


Figure 4.6 **A:** The effect of repeated administration of MDMA (3 or 6 mg/kg i.p 3 injections every 2 h) on body temperature (mean ± SEM, n = 8 per treatment) measured using a rectal probe thermometer. Rectal temperature was measured 2 h after the last injection (t = 360). **B:** Body temperature data obtained from the experiment in chapter 3 using radiotelemetry to compare the results from two experiments using the same treatment but different measuring devices. Data are obtained from the same time point which is 2 h following the last injection (t = 360). *$p < 0.05$ compared to saline, $^{\$}$ $p < 0.05$, $^{\$\$}$ $p < 0.01$ compared to MDMA (3 x 3 mg/kg) (Tukey's *post hoc* test following one-way ANOVA).

(d) Locomotor activity on day 14

Rats were habituated in the infra-red activity monitor chamber 13 days after MDMA or saline treatment. All three groups of rats exhibited very similar ambulatory and fine movement profiles with activity decreasing across the 60 min period of habituation (Figure 4.7). Two-way ANOVA showed a significant time effect on ambulatory activity ($F(11,231) = 64.73$, $p < 0.0001$) and fine activity ($F(11,231) = 13.24$, $p < 0.0001$). There was no effect of treatment on ambulatory activity as analysed by two-way ANOVA. However a significant treatment effect on fine activity was shown using two-way ANOVA ($F(2,231) = 3.57$, $p = 0.0461$). Data, calculated as total activity in 60-min test period, showed that rats treated with the higher dose of MDMA (3 x 6 mg/kg) exhibited an increase of total fine activity which was significantly different from saline ($p < 0.05$; Tukey's *post hoc* test following one-way ANOVA) (Figure 4.7B).

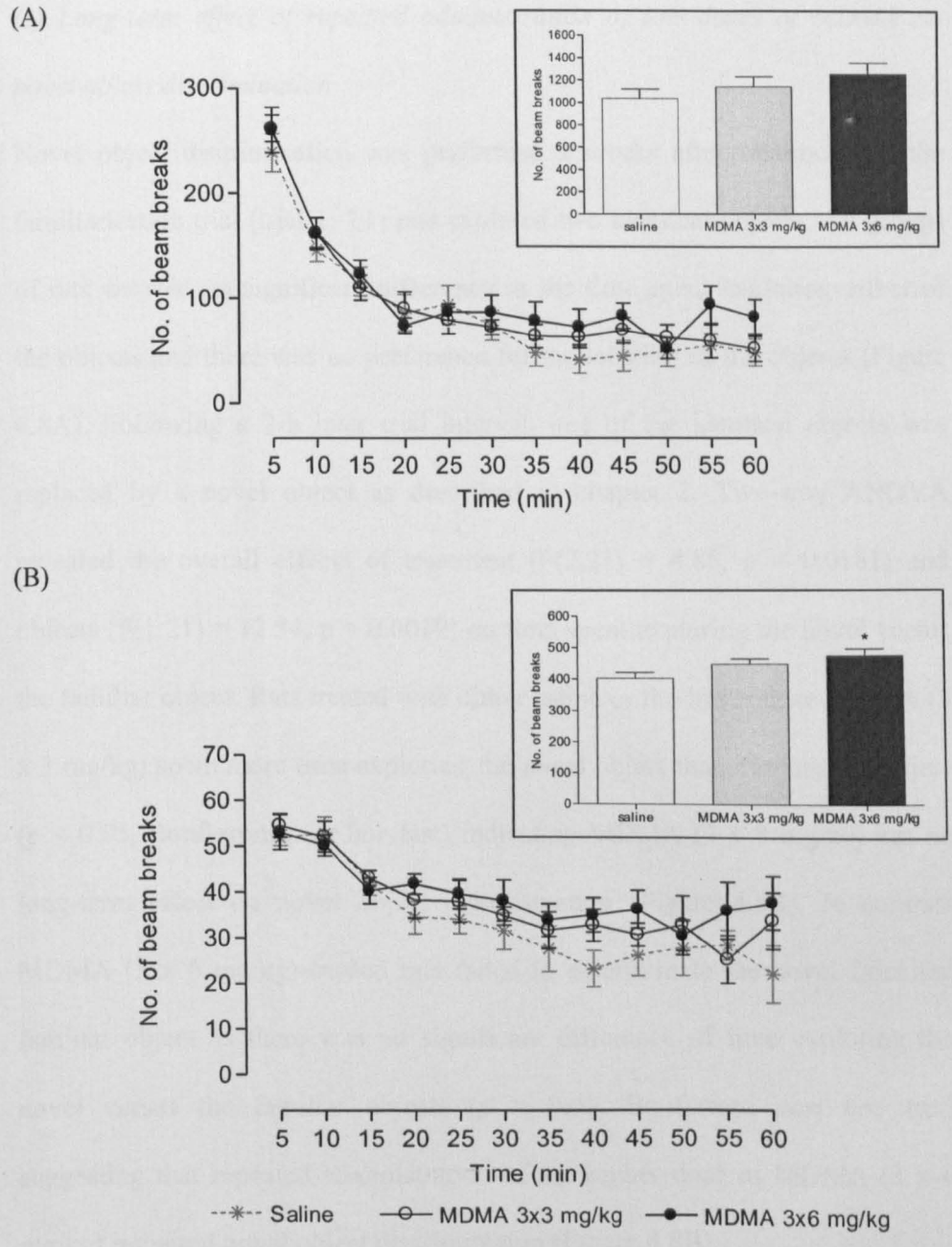


Figure 4.7 Effects of MDMA (3 or 6 mg/kg i.p.) or saline (1 ml/kg i.p.) x 3 on **A:** ambulatory movements and **B:** fine movements 13 days after treatment using a computer-controlled infra-red activity monitor chamber. Data are expressed as 5-min time bin and as total values over a 60 min test period (the inset figures) (mean ± SEM, n = 8 per group). * $p < 0.05$ compared to saline (Tukey's *post hoc* test following one-way ANOVA).

(e) Long-term effect of repeated administration of low doses of MDMA on novel object discrimination

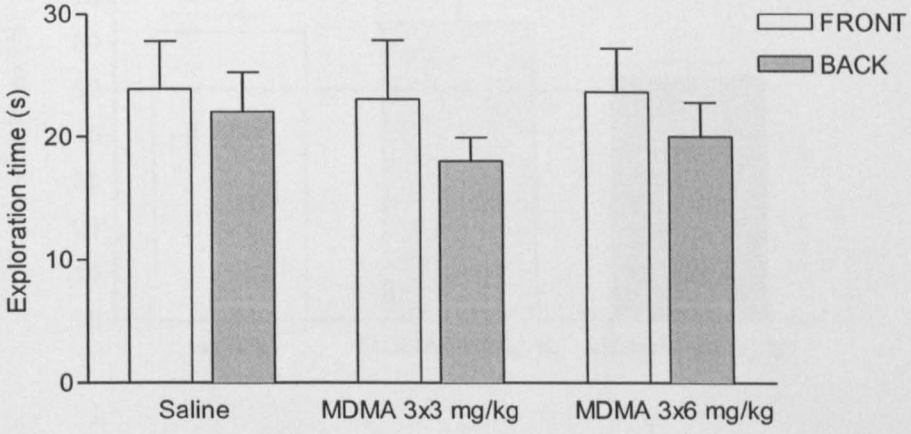
Novel object discrimination was performed 2 weeks after treatment. In the familiarisation trial (trial 1; T1) rats explored two identical objects. All groups of rats showed no significant difference in the time spent exploring either of the objects and there was no preference for the location of the objects (Figure 4.8A). Following a 2-h inter trial interval, one of the identical objects was replaced by a novel object as described in chapter 2. Two-way ANOVA revealed the overall effects of treatment ($F(2,21) = 4.88$, $p = 0.0181$) and objects ($F(1,21) = 12.54$, $p = 0.0019$) on time spent exploring the novel versus the familiar object. Rats treated with either saline or the lower dose MDMA (3 x 3 mg/kg) spent more time exploring the novel object than the familiar object ($p < 0.05$, Bonferroni *post hoc* test) indicating MDMA (3 x 3 mg/kg) has no long-term effect on novel object discrimination (Figure 4.8B). In contrast MDMA (3 x 6 mg/kg)-treated rats failed to discriminate the novel from the familiar object as there was no significant difference of time exploring the novel versus the familiar objects ($p > 0.05$, Bonferroni *post hoc* test) suggesting that repeated administration of the higher dose of MDMA (3 x 6 mg/kg) impaired novel object discrimination (Figure 4.8B).

The time spent exploring the novel versus the familiar objects in the choice trial was converted into a preference index (PI) (e.g. [novel object/ novel object + familiar object] x 100) and shown in Figure 4.9. The PI value of MDMA (3 x 3 and 3 x 6 mg/kg)-treated rats was not significantly different from saline-treated rats ($p > 0.05$, unpaired Student *t*-test). The PI values of saline were

significantly greater than a PI value of 50% chance level ($p = 0.0122$, one sample *t*-test) (Figure 4.9). Although rats treated with MDMA (3 x 3 mg/kg) spent more time exploring the novel than the familiar object ($p < 0.05$, Bonferroni *post hoc* test) (Figure 4.8B), the PI value of MDMA (3 x 3 mg/kg) treated rat was not significantly different from the 50% chance level ($p = 0.0519$, one sample *t*-test) (Figure 4.9). Moreover the PI value of MDMA (3 x 6 mg/kg) treated rats was not significantly different from 50% chance level ($p = 0.4102$, one sample *t*-test) (Figure 4.9).

The total time spent by the rats exploring two identical objects in trial 1 (Total T1 time) and the total time rats spent exploring the novel and the familiar object in trial 2 (total T2 time) are presented in Table 4.1. While there was no significant difference in total T1 time between the three groups, rats treated with MDMA (3 x 6 mg/kg) had significantly lower total T2 time activity compared to saline controls ($p < 0.05$; Tukey's *post hoc* test following one-way ANOVA) (Table 4.1).

(A)



(B)

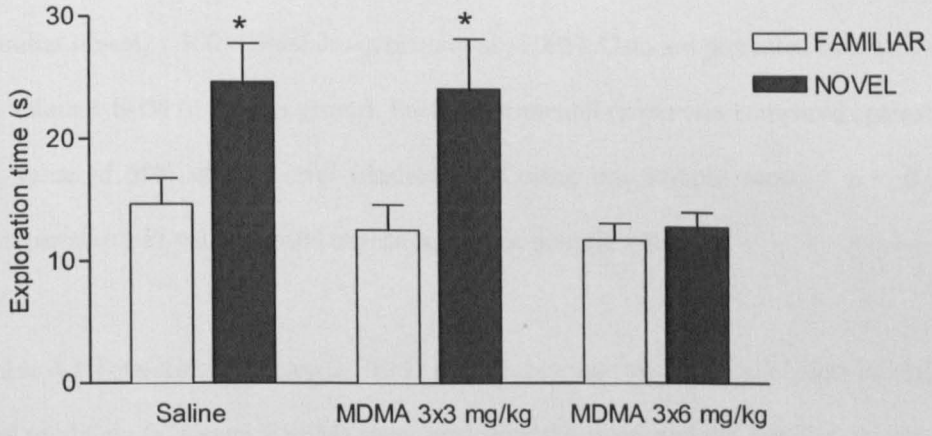


Figure 4.8 Effects of MDMA on the time spent (s, means ± SEM) exploring **A:** each of the identical objects during trial 1 and **B:** the novel versus the familiar object during trial 2 which was 2 h after trial 1. Rats received either MDMA (3 or 6 mg/kg i.p.) or saline (1 ml/kg i.p.) 3 times every 2 h (n = 8 per treatment). * $p < 0.05$ compared with time spent at the familiar object in the same treatment group (Bonferroni *post hoc* test).

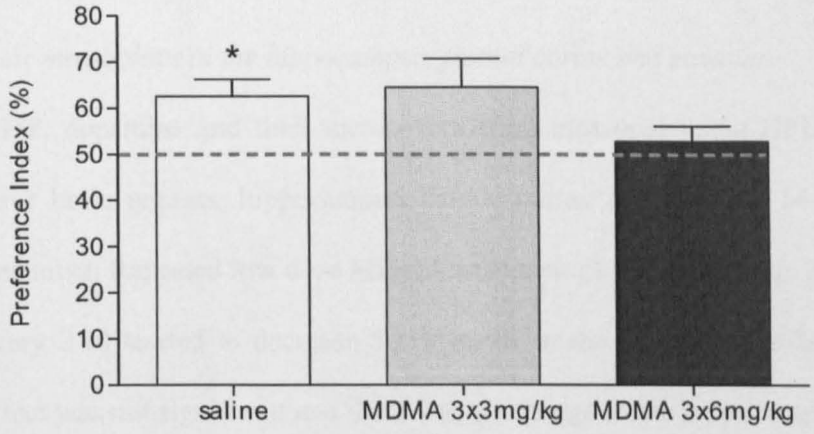


Figure 4.9 Time spent exploring the novel versus the familiar objects in the choice trial was converted into a preference index (PI) (e.g. [novel object/ novel object + familiar object] x 100) (Bruel-Jungerman et al., 2005). Data are presented as means of PI values ± SEM (n = 8 per group). Each experimental group was compared against a PI value of 50% chance level (dashed line) using one sample *t*-test. * $p < 0.05$ compared to a PI value of 50% chance level (one-sample *t*-test)

Table 4.1 Total time (s, means ± SEM) spent exploring two identical objects in trial 1 and total time (s, means ± SEM) spent exploring the novel and the familiar objects in trial 2.

Treatment	Total T1 time (s, mean ± SEM)	Total T2 time (s, mean ± SEM)
saline	46 ± 6	39 ± 4
MDMA (3 x 3 mg/kg)	41 ± 6	37 ± 4
MDMA (3 x 6 mg/kg)	44 ± 5	25 ± 2*

* $p < 0.05$ compared with saline in trial 2 (Tukey's *post hoc* test following one-way ANOVA)

(f) Long-term effects of repeated low doses of MDMA on 5-HT, dopamine and their metabolites in the hippocampus, frontal cortex and striatum

5-HT, dopamine and their metabolites were measured using HPLC-ECD in three brain regions; hippocampus, frontal cortex and striatum 14 days after treatment. Repeated low dose MDMA treatment (3 or 6 mg/kg i.p. 3 injections every 2 h) tended to decrease 5-HT levels in the hippocampus however the effect was not significant and there was no change in the hippocampal 5-HIAA levels following MDMA treatment (Table 4.2). There were no significant differences in either 5-HT or 5-HIAA levels in the frontal cortex or the striatum (Table 4.2). Moreover MDMA tended to increase dopamine, DOPAC and HVA levels in the striatum however these effects were again not significant (Table 4.3).

Table 4.2 No long-term effect of repeated administration of low doses of MDMA on 5-HT and 5-HIAA levels in the hippocampus, frontal cortex and striatum. Data are presented as mean of concentration (pmol/mg of tissue) ± SEM (n = 8 per group).

	Hippocampus		Frontal cortex		Striatum	
Treatment	5-HT	5-HIAA	5-HT	5-HIAA	5-HT	5-HIAA
Saline	1.92±0.13	1.94±0.14	2.80±0.32	1.44±0.09	3.81±0.29	2.71±0.14
MDMA (3x3 mg/kg)	1.82±0.08	1.92±0.08	2.90±0.23	1.35±0.13	3.60±0.32	2.37±0.19
MDMA (3x6 mg/kg)	1.78±0.11	1.94±0.09	2.74±0.29	1.45±0.14	3.62±0.32	2.47±0.12

Table 4.3 No long-term effect of repeated administration of low doses of MDMA on dopamine, DOPAC and HVA levels in the striatum. Data are presented as mean of concentration (pmol/mg of tissue) ± SEM (n = 8 per group).

Treatment	Dopamine	DOPAC	HVA
Saline	27.27 ± 4.57	4.06 ± 0.58	2.09 ± 0.37
MDMA (3x3 mg/kg)	31.34 ± 3.26	4.44 ± 0.38	2.32 ± 0.27
MDMA (3x6 mg/kg)	37.64 ± 4.97	5.61 ± 0.55	2.85 ± 0.38

4.1.4 Discussion

The first experiment of this chapter aimed to determine the long-term effects of repeated administration of low doses of MDMA on novel object discrimination and changes in 5-HT, dopamine and their metabolites in the hippocampus, striatum and frontal cortex. The experimental procedure used in this experiment was similar to that used in the previous chapter as the rats were singly housed in a computer-controlled infra-red activity monitor chamber during treatment and the acute effect of the MDMA treatment on activity were measured. In addition rectal temperature was measured 2 h after the last injection of the drugs. Novel object discrimination and changes in amine neurotransmitters were measured 2 weeks after treatment.

The activity response to MDMA was measured during treatment using the computer-controlled infra-red activity monitor chambers which provided data of 'ambulatory' and 'fine' movements. The ambulatory movement data represented locomotor activity of the rats and repeated administration of the higher dose of MDMA (3 x 6 mg/kg) produced a marked and sustained hyperactivity, similar to that previously demonstrated in chapter 3 using radiotelemetry. The lower dose of MDMA (3 x 3 mg/kg) produced little effect on locomotor activity after the second and third injections which was similar to the radiotelemetry measurement in chapter 3 when there was no effect on activity with lower dose of MDMA.

Unlike the data from radiotelemetry, the computer-controlled infra-red activity monitor chamber provides data of 'fine' movements which were counted when

the rats repeatedly disrupted the same beam. It was demonstrated in the present study that both doses of MDMA markedly increased fine movement after each injection. MDMA-induced hyperactivity has been generally reported (Colado et al., 1993, Shankaran and Gudelsky, 1999, Spanos and Yamamoto, 1989). The quality of hyperactivity produced by MDMA in rats is unique as MDMA produces both amphetamine-like hyperlocomotor activity together with the symptoms of the serotonin syndrome, which include low body posture, side-to-side headweaving, lateral forepaw treading and the autonomic signs of piloerection and salivation (Gold et al., 1988, Spanos and Yamamoto, 1989). In the present study the data for the fine movement was counted when the rats repeatedly disrupted the same beam and therefore probably represent the symptoms of the serotonin syndrome, especially side-to-side headweaving, which would be counted as fine movements. The increase in fine movements following both doses of MDMA is indicative of the serotonin syndrome following MDMA. Interestingly the increase in fine body movements with the higher dose persisted on day 14 after treatment suggesting MDMA treatment can have long-term effects on fine motor function.

Body temperature was measured 2 h after the last injection showing that the higher dose of MDMA (3 x 6 mg/kg) slightly increased body temperature by +0.5 °C while the lower dose of MDMA (3 x 3 mg/kg) decreased body temperature by -0.3 °C compared to saline control. The pattern of body temperature change was comparable to those measured in the previous chapter using radiotelemetry but the magnitude of increase in the present study by the higher dose of MDMA (6 mg/kg) was lower (+0.5 °C using rectal thermometer

probe compared to +1.7 °C by radiotelemetry). It might be due to the physical stress state of the rats in radiotelemetry experiment being higher as rats had been implanted with microdialysis probe the day before MDMA administration. This higher physical stress might have affected temperature response to the drug.

The main finding of the present study is that repeated administration of 6 but not 3 mg/kg MDMA (3 times every 2 h) disrupted novel object discrimination 2 weeks after treatment (Figure 4.8). The effect at 3 mg/kg did not show in the novel object PI value (Figure 4.9) suggesting only a marginal novel object discrimination at this dose. The long-term effect of MDMA administration on novel object recognition seems to vary greatly across studies. McGregor et al (2003) showed a disruption of novel object recognition with a 1 h inter-trial interval 10-12 weeks following repeated MDMA administration (4 x 5 mg/kg for 2 days) treatment while Morley et al (2001) reported a significant deleterious effect on novel object recognition with a 15 min but not 1 h inter-trial interval 14 weeks after MDMA administration (4 x 5 mg/kg for 2 days). On the other hand Able et al (2006) and Skelton et al (2008) gave MDMA (4 x 15 mg/kg) to the rats and produced no impairment of novel object recognition with a 1 h inter-trial interval 4-6 weeks after treatment.

The results from the previous studies are difficult to compare with the present study as there were differences in the doses used, dosage regimen and the experimental protocol. However the present study gave repeated low doses of MDMA (3 x 6 mg/kg accumulating to 18 mg/kg over 4 h) and the studies by

Morley et al (2001) and McGregor et al (2003) also gave repeated low dose of MDMA (4 x 5 mg/kg for 2 days accumulating to 40 mg/kg) and all these studies identified long-term disruption of novel object recognition while studies by Able et al (2006) and Skelton et al (2008) using repeated high dose of MDMA (4 x 15 mg/kg accumulating to 60 mg/kg) showed no impairment of novel object recognition. The experimental protocols including the inter-trial intervals and the duration of the novel object recognition task after treatment also vary across the studies. Morley et al (2001) showed that disruption of novel object recognition depended on the inter-trial interval with impairment with a 15 min delay but not 1 h delay while the present study with a 2 h delay found disruption. In addition novel object recognition was performed 2 weeks after MDMA treatment in the present study while others performed the novel object recognition task 4-6 weeks (Able et al., 2006, Skelton et al., 2008) or over 3 months (McGregor et al., 2003, Morley et al., 2001) after MDMA treatment. Overall however the evidence suggests that "binge-type" repeated MDMA treatment in the rat can lead to long-term disruption to novel object discrimination indicating the impairment of working as well as recognition memory following repeated MDMA treatment in the rats. In humans there have been a number of studies showing impairments of working memory following MDMA use especially in heavy and chronic recreational users (Fox et al., 2002, Gouzoulis-Mayfrank et al., 2000, McCann et al., 1999b, Morgan et al., 2002, Wareing et al., 2000). The present study suggests the risk of impairment of working memory following binge use of MDMA.

Rats treated with the higher dose of MDMA (3 x 6 mg/kg) showed a significantly lower total exploratory time in trial 2 compared to saline and the lower dose of MDMA (3 x 3 mg/kg) but a significant increase in fine movements as already discussed. This increase in fine movements might interfere with performance of the novel object recognition task however further investigation is required.

The evidence for an involvement of 5-HT neurotoxicity on memory impairment following MDMA treatment is controversial. McGregor et al (2003) reported an impairment of novel object recognition with decreased 5-HT in the hippocampus, striatum, amygdala and cortex. In contrast Able et al (2006) and Skelton et al (2008) found decreased 5-HT in the hippocampus, neostriatum and frontal cortex but no impairment of novel object recognition. The present study showed a disruption of novel object recognition 2 weeks following MDMA (3 x 6 mg/kg) administration without changes in 5-HT in the hippocampus, striatum and frontal cortex, indicating that the long-term impairment of novel object discrimination caused by MDMA did not depend upon distribution of 5-HT function.

The long-term depletion of brain 5-HT following MDMA administration has been well documented especially after high or repeated doses of MDMA to the rats (see review Capela et al., 2009). There was however no change in 5-HT and 5-HIAA in hippocampus, striatum and frontal cortex 2 weeks following repeated low doses of MDMA in the present study. A similar study by Baumann et al (2007) also showed that low dose of MDMA (3 x 1.5 mg/kg)

had no effect on 5-HT in the frontal cortex and striatum 2 weeks after administration while a higher dose of MDMA (3 x 7.5 mg/kg) produced 50% reduction of 5-HT in those brain regions. The hyperthermic response has been previously shown to play a role in MDMA-induced neurotoxicity (Baumann et al., 2008a, Green et al., 2004, Malberg and Seiden, 1998, Sanchez et al., 2004). Recently Baumann et al (2008a) showed a negative correlation between the acute hyperthermic response and 5-HT depletion 2 weeks after MDMA (3 x 7.5 mg/kg). In the present study there was no increase in body temperature 2 h after the last injection of the lower dose of MDMA (3 x 3 mg/kg; -0.3 °C) and only a small increase after the higher dose (3 x 6 mg/kg; +0.5 °C) and no consequent changes in 5-HT and 5-HIAA levels 2 weeks after treatment further suggesting 5-HT loss may have a link with the degree of hyperthermia produced by a given dose of MDMA.

In summary, the present study provided evidence of long-term disruption of novel object discrimination following "binge-type" repeated MDMA administration (3 x 6 mg/kg) and that these impairments of recognition and working memory are not directly related to changes in brain region levels of 5-HT or dopamine. The hyperthermic response is thought to be a factor influencing MDMA-induced 5-HT neurotoxicity but the doses given in this experiment did not produce significant hyperthermia. However it is known that single housing may influence the hyperthermic effect of MDMA as discussed in chapter 3. Thus the next experiment in this chapter compared the temperature response and MDMA-induced 5-HT neurotoxicity in singly and group housed rats.

4.2. Experiment 2: Acute effects of group housing on the MDMA-induced change in body temperature and the long-term effects on novel object discrimination and neurotoxicity

4.2.1 Introduction

It was demonstrated in the first experiment of this chapter that repeated administration of the higher dose of MDMA (3 x 6 mg/kg) slightly increased body temperature 2 h after the last injection. Studies using radiotelemetry in the previous chapter found that this dose of MDMA (3 x 6 mg/kg) impaired thermoregulation in the rats. However an increase in body temperature was not sufficient to produce neurotoxicity in the present study. In chapter 3 the importance of housing conditions on MDMA-induced hyperthermia and subsequent 5-HT neurotoxicity was discussed as aggregation has been previously reported to potentiate the behavioural and lethal effects of stimulants including amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA) and MDMA in animals (Chance, 1947, Davis and Borne, 1984, Fantegrossi et al., 2003, Greenblatt and Osterberg, 1961). The mechanism of toxicity has generally thought to be directly related to raised body temperature (Askew, 1961, Craig and Kupferberg, 1972) however no study has compared the effect of single and group housing condition on the body temperature response and subsequent neurotoxicity produced by MDMA. In addition investigation into the aggregate toxicity of MDMA is particularly relevant due to the common condition of social MDMA use by humans (Green et al 2003). It is hypothesized in the present study that

the condition of crowding could produce greater hyperthermia and thus potentiate the neurotoxic effects of MDMA.

The aims of the second experiment of this chapter were to compare the effects of single and group housing conditions on MDMA-induced hyperthermia and determine the involvement of hyperthermia on MDMA-induced 5-HT neurotoxicity. The long-term effect of repeated MDMA administration on novel object discrimination was also examined. The higher dose of MDMA (3x 6 mg/kg) was selected as it had disrupted novel object discrimination in *experiment 4.1.*

4.2.2 Materials and methods

Animal

Adult male Lister hooded rats (Biomedical Service Unit, University of Nottingham, UK) weighing 300-350 g were housed on a 12 h light/dark cycle (light on at 7.00h) in constant room temperature (21±2 °C) and humidity (45-65%). Rats had free access to food and water. All experiments were performed in accordance with the UK Animals (Scientific Procedures) Act, 1986 under project license 40/2715 and approved by the University of Nottingham Local Ethical Review Committee.

Experimental procedure

Rats were housed in groups of 4 throughout the experiment. One day before treatment each rat was placed in an individual infra-red activity monitor chamber to measure activity baseline for each group as in *experiment 4.1.* On the treatment day rats were injected with either MDMA (6 mg/kg i.p., (±)-

MDMA HCl, Sigma, UK) or saline (1 ml/kg i.p.) 3 times every 2 h while still group housed. Rectal temperature was measured 30, 60 min before the first injection then every 30 min until 2 h after the last injection using a digital thermocouple probe thermometer (Portec Instrumentation Ltd, P9005) inserted 6-8 cm into the rectum. On day 14 each rat was habituated in the individual infra-red activity monitor chamber and activity recorded for 1 h. Novel object discrimination was performed on day 15. Brain tissue was dissected for later analysis of 5-HT, dopamine and their metabolites as previously described.

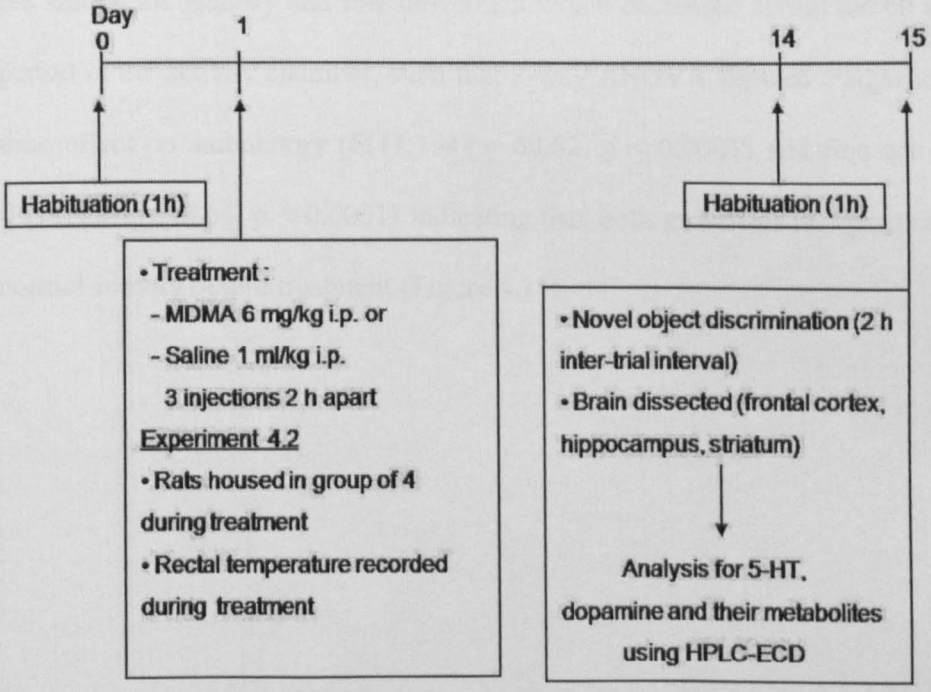


Figure 4.10 Experimental procedure to determine the effects of repeated low dose MDMA administration while rats were group housed during treatment on body temperature, long-term effect of MDMA on novel object discrimination and changes in brain amines.

Statistical analysis

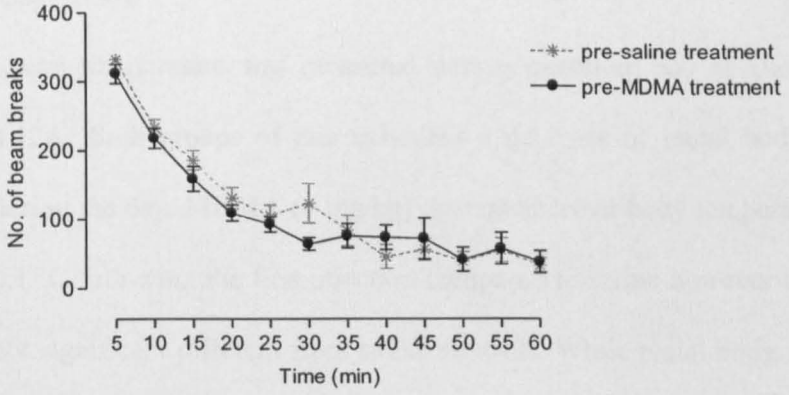
Two-way ANOVA followed by Bonferroni *post hoc* test, one-way ANOVA followed by Tukey's *post hoc* test, unpaired Student *t*-test and one sample *t*-test were used where appropriate. $P < 0.05$ was considered as a significant difference.

4.2.3 Results

(a) Locomotor activity before treatment

Similar to the first experiment both pre-saline and pre-MDMA rats exhibited the similar ambulatory and fine movement which decreased across the 60 min period in the activity chamber, such that 2-way ANOVA showed a significant time effect on ambulatory ($F(11,154) = 60.62$, $p < 0.0001$) and fine activity ($F(11,154) = 16.64$, $p < 0.0001$) indicating that both groups of rats performed normal activity before treatment (Figure 4.11).

(A)



(B)

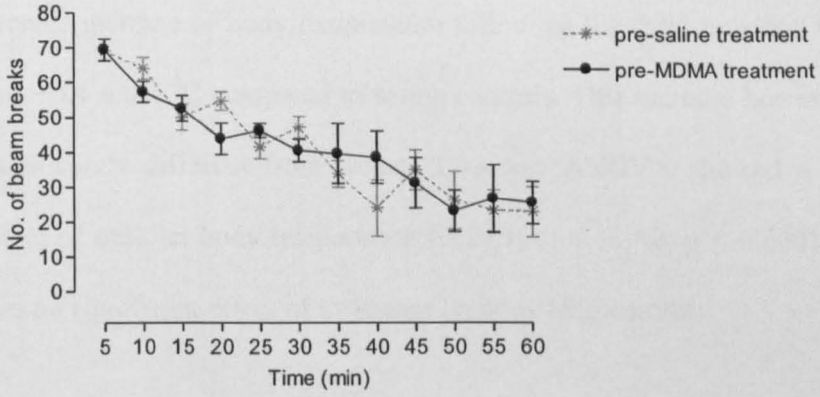


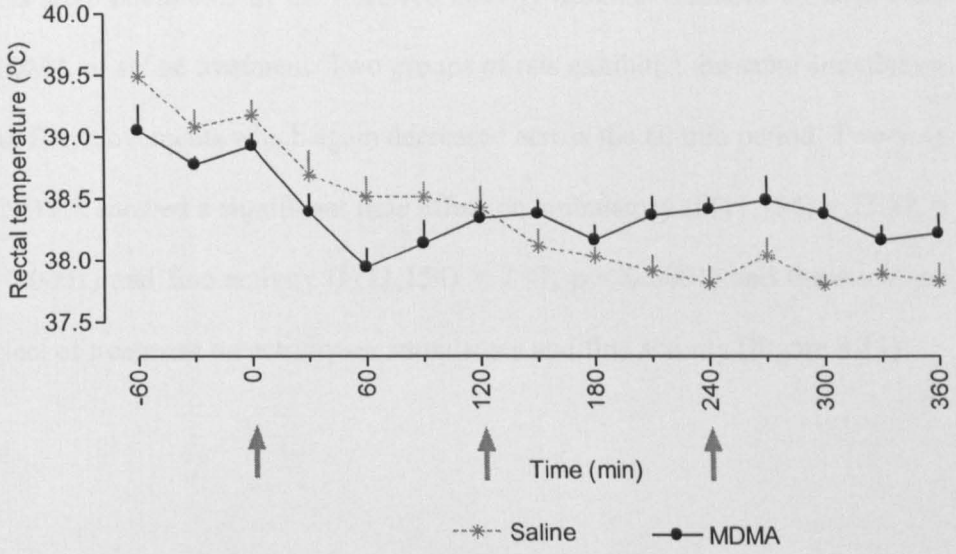
Figure 4.11 **A**: Ambulatory and **B**: fine activity 1 day before treatment. Each point represents mean of number of beam breaks in 5-min ± SEM (n = 8 per group).

(b) The acute effect of repeated administration of low dose of MDMA on body temperature

Rectal temperature was measured during treatment day as shown in Figure 4.12A. Both groups of rats exhibited a decrease of rectal body temperature during the day. MDMA (6 mg/kg) decreased rectal body temperature by -0.3 ± 0.1 °C following the first injection compared to saline however this effect was not significant different from saline controls. While rectal body temperature in saline-treated rats continuously decreased after the second and the third injections, MDMA (6 mg/kg) tended to increase body temperature. The average increase of body temperature following the third injection of MDMA was +0.4 ± 0.1 °C compared to saline controls. This increase however was not significantly different from saline. Two-way ANOVA showed a significant effect of time on body temperature ($F(14,196) = 19.80$, $p < 0.0001$) but there was no significant effect of treatment on body temperature.

Figure 4.12 also shows the comparison between the results from the present experiment, using a rectal probe thermometer (Figure 4.12A), and the experiment in chapter 3 (Figure 4.12B) where radiotelemetry was used. Data obtained from radiotelemetry showed that repeated MDMA administration (3 x 6 mg/kg) decreased body temperature after the first injection then body temperature continuously increased after the second and the third injection (Figure 4.12B) with an average increase of +0.9 ± 0.3 °C after the last injection compared to saline controls (Figure 4.12B).

(A)



(B)

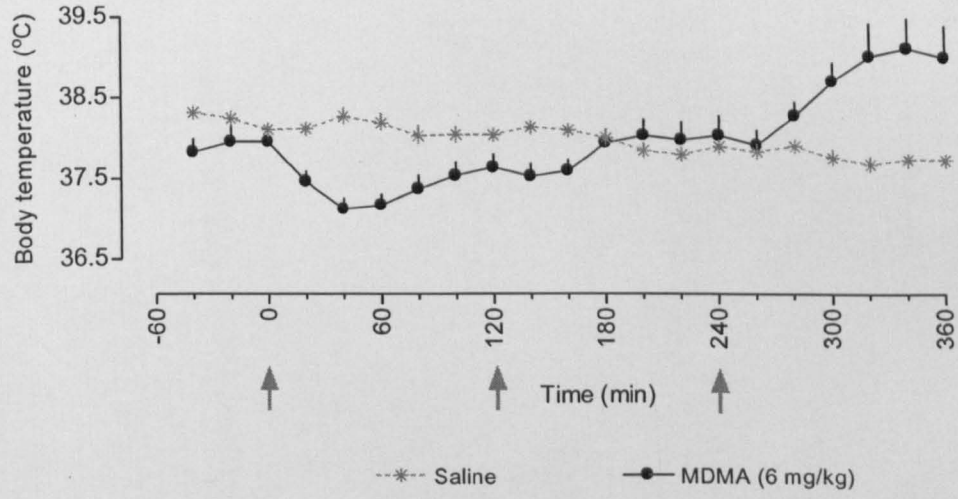


Figure 4.12 **A:** Effect of repeated administration of either MDMA (6 mg/kg) or saline 3 injections given 2 h intervals on body temperature (injections indicated by arrows). Rectal temperature was measured using a rectal probe every 30 min (means ± SEM, n = 8 per group). **B:** Body temperature data obtained from the experiment in chapter 3 using radiotelemetry to compare the results from two experiments using the same treatment but different measuring devices. Data are presented as means of body temperature in 20-min time bins ± SEM (injections indicated by arrows, n = 6 per group).

(c) Locomotor activity on day 14

Rats were habituated in the infra-red activity monitor chamber 13 days after MDMA or saline treatment. Two groups of rats exhibited the same ambulatory and fine movements which again decreased across the 60 min period. Two-way ANOVA showed a significant time effect on ambulatory ($F(11,154) = 23.33$, $p < 0.0001$) and fine activity ($F(11,154) = 7.97$, $p < 0.0001$) and there was no effect of treatment on activity on ambulatory and fine activity (Figure 4.13).

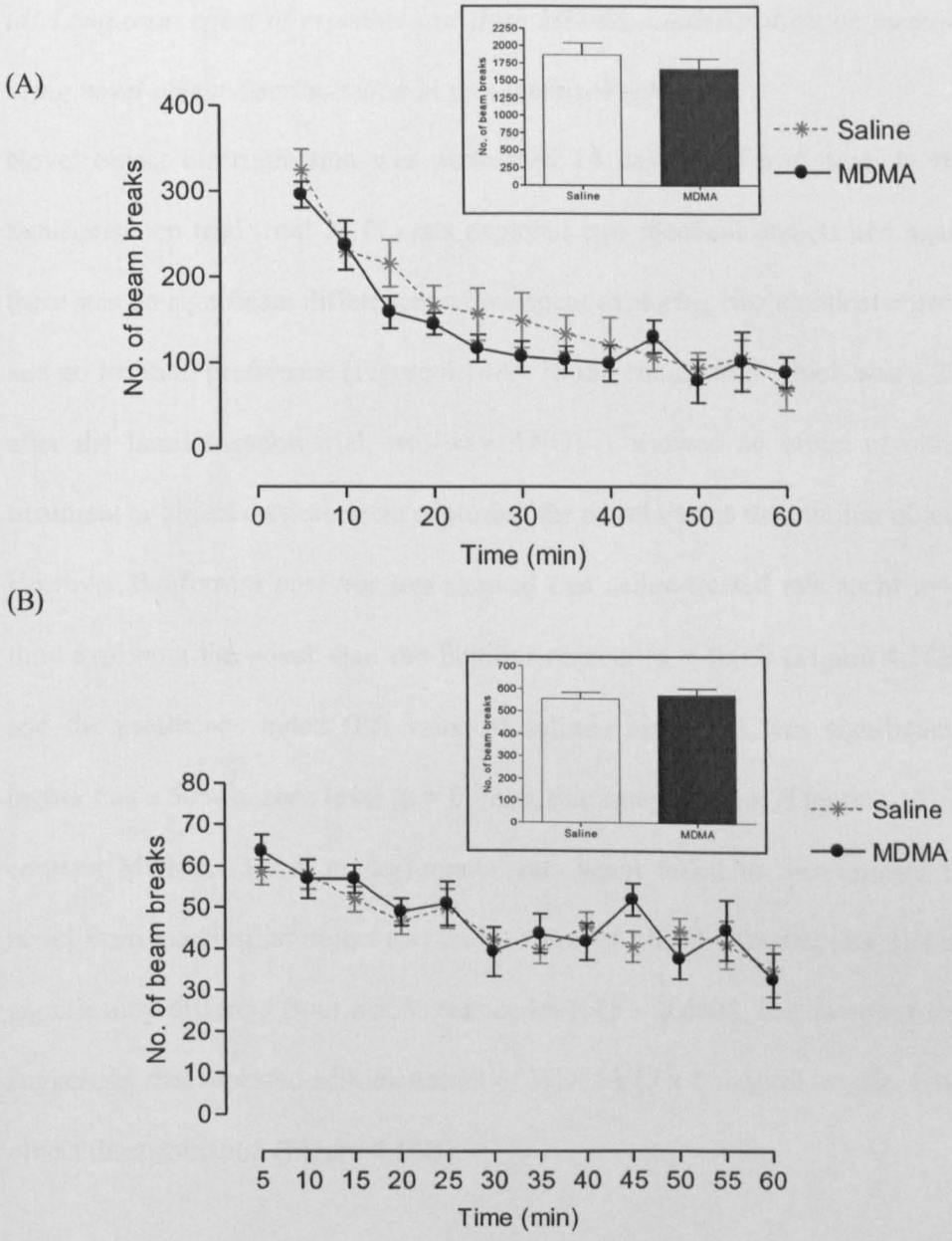


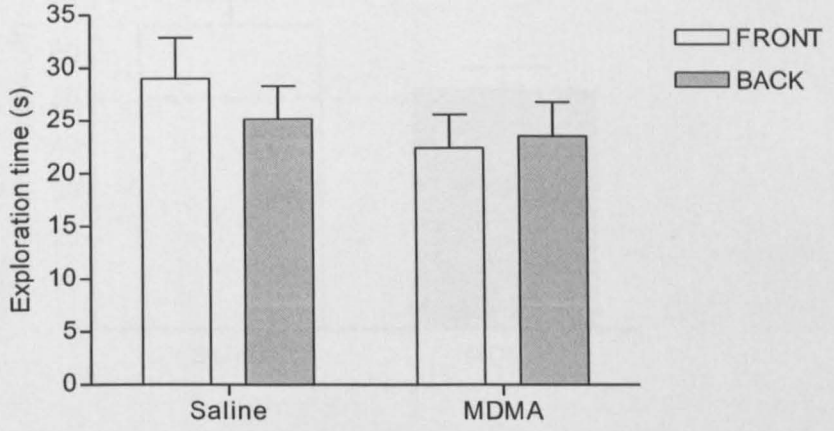
Figure 4.13 Effects of MDMA (3 x 6 mg/kg) or saline on **A**: ambulatory and **B**: fine movements 13 days after treatment. Data are expressed as 5-min time bins and as total values over 60 min test period (the inset figures) in the infra-red activity monitor chamber (mean ± SEM, n = 8 per group).

(d) Long-term effect of repeated low dose MDMA administration on memory using novel object discrimination in group housed rats

Novel object discrimination was performed 14 days after treatment. In the familiarisation trial (trial 1; T1) rats explored two identical objects and again there was no significant difference in time spent exploring two identical objects and no location preference (Figure 4.14A). In the choice trail which was a 2 h after the familiarisation trial, two-way ANOVA showed no effect of either treatment or object on time spent exploring the novel versus the familiar object. However Bonferroni *post hoc* test showed that saline-treated rats spent more time exploring the novel than the familiar objects ($p < 0.05$) (Figure 4.14B), and the preference index (PI) value of saline-treated rats was significantly higher that a 50% chance level ($p = 0.0260$, one sample *t*-test) (Figure 4.15). In contrast MDMA (3 x 6 mg/kg)-treated rats again failed to discriminate the novel from the familiar object and the PI value of MDMA-treated rats was not significantly different from a 50% chance level ($p = 0.6683$, one sample *t*-test) suggesting that repeated administration of MDMA (3 x 6 mg/kg) impairs novel object discrimination (Figure 4.14B).

Two-way ANOVA showed no effect of trial and treatment on total exploration time. However the total exploration time in trial 2 (total T2 time) was decreased in saline-treated rats compared to total exploration time in trial 1 (total T1 time) ($p < 0.05$; Student unpaired *t*-test) (Table 4.4).

(A)



(B)

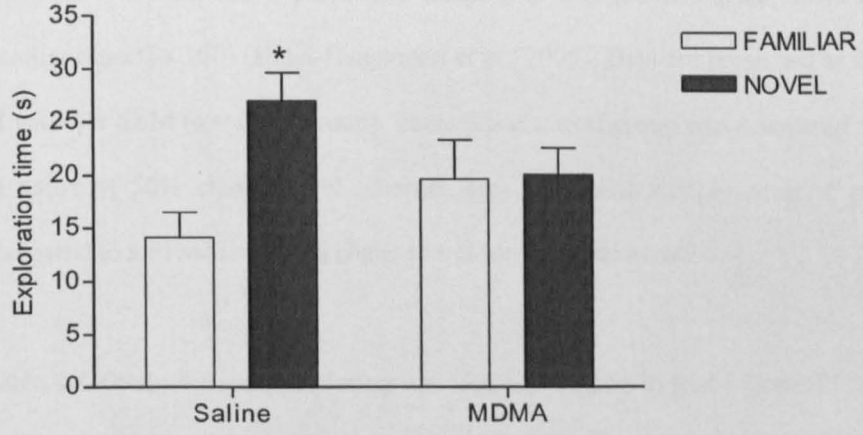


Figure 4.14 Effect of MDMA on the time spent (s, means ± SEM) exploring **A**: each of the identical objects during trial 1 and **B**: the novel versus the familiar objects during trial 2 which was 2 h after trial 1. Rats received either MDMA (6 mg/kg i.p.) or saline (1 ml/kg i.p.) 3 times every 2 h (n = 8 per treatment) and performed novel object discrimination 14 days after treatment. * $p < 0.05$ compared with time spent at the familiar object in the same treatment group (Bonferroni *post hoc* test).

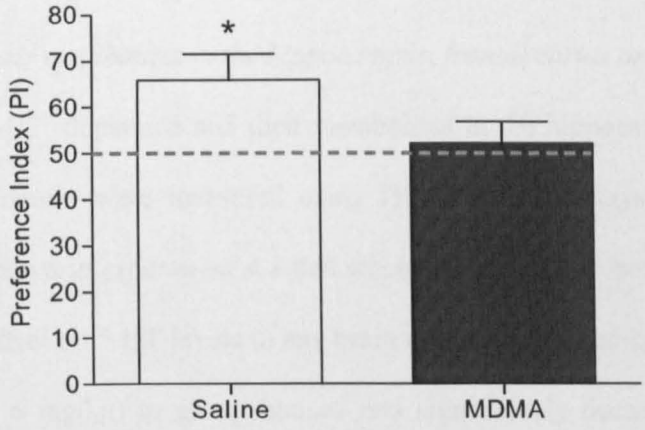


Figure 4.15 Time spent exploring the novel versus the familiar objects in the choice trial was converted into a preference index (PI) (e.g. [novel object/ novel object + familiar object] x 100) (Bruel-Jungerman et al., 2005). Data are presented as means of PI values ± SEM (n = 8 per group). Each experimental group was compared against a PI value of 50% chance level (dashed line) using one sample *t*-test. * $p < 0.05$ compared to a PI value of 50% chance level (one-sample *t*-test)

Table 4.4 Total time spent exploring two identical objects in trial 1 (total T1 time) and total time spent exploring the novel and the familiar objects in trial 2 (total T2 time) (s, means ± SEM, n = 8 per group).

Treatment	Total T1 time (s, mean ± SEM)	Total T2 time (s, mean ± SEM)
saline	54 ± 6	41 ± 2*
MDMA (3 x 6 mg/kg)	45 ± 6	40 ± 4

* $p < 0.05$ compared to saline in trial 1

(e) Long-term effects of repeated low doses of MDMA on 5-HT, dopamine and their metabolites in the hippocampus, frontal cortex and striatum

5-HT, dopamine and their metabolites in the hippocampus, frontal cortex and striatum were measured using HPLC-ECD 14 days after treatment. It was shown in *experiment 4.1* that when given to singly housed rats MDMA had no effect on 5-HT levels in any brain regions examined however given MDMA (3 x 6 mg/kg) to group housed rats significantly decreased 5-HT levels in the hippocampus ($p = 0.0113$, unpaired Student *t*-test) (Table 4.5). There was no change in hippocampal 5-HIAA levels following MDMA administration and also no significant difference of either 5-HT or 5-HIAA levels in the frontal cortex or the striatum (Table 4.5). MDMA tended to increase dopamine, DOPAC and HVA levels in the striatum however this was not significantly different from saline controls (Table 4.6).

Table 4.5 The long-term effects of repeated administration of low dose of MDMA on 5-HT and 5-HIAA levels in the hippocampus, frontal cortex and striatum. Data are presented as mean of concentration (pmol/mg of tissue) ± SEM (n = 8 per group).

	Hippocampus		Frontal cortex		Striatum	
Treatment	5-HT	5-HIAA	5-HT	5-HIAA	5-HT	5-HIAA
Saline	2.19±0.08	2.07±0.04	2.52±0.08	1.34±0.06	3.11±0.17	2.53±0.16
MDMA	1.77±0.11*	1.95±0.12	2.34±0.19	1.29±0.08	2.80±0.27	2.24±0.08

*p = 0.0113, unpaired Student *t*-test

Table 4.6 Long-term effects of repeated administration of low dose of MDMA on dopamine, DOPAC and HVA levels in the striatum. Data are presented as mean of concentration (pmol/mg of tissue) ± SEM (n = 8 per group).

Treatment	Concentration (pmol/mg of tissue)		
	Dopamine	DOPAC	HVA
Saline	26.81 ± 4.11	3.38 ± 0.51	1.68 ± 0.30
MDMA	29.71 ± 2.36	3.61 ± 0.27	2.00 ± 0.14

4.2.4 Discussion

The second experiment of this chapter aimed to determine the effect of the housing conditions on MDMA-induced changes in body temperature and long-term 5-HT neurotoxicity. Rats were group housed during MDMA treatment to produce aggregation as it was previously reported that there was an increase in the behavioural and lethal effects of amphetamines, including MDMA, in group housed animals (Chance, 1947, Davis and Borne, 1984, Fantegrossi et al., 2003). The main finding of the present study was that the pattern of body temperature change following repeated MDMA administration in the group housed rats, using a rectal probe, was similar to that seen in the singly housed rats in chapter 3, using radiotelemetry to measure body temperature (Figure 4.12) indicating that there was no aggregation toxicity on hyperthermic effect of MDMA in rats. However although the pattern of hyperthermic response to MDMA of singly and group housed rats is not different, the present study showed that group housed rats significantly decreased 5-HT levels in the hippocampus 2 weeks after repeated MDMA administration (3 x 6 mg/kg) *(experiment 4.2)* while the singly housed rats showed no long-term change in 5-HT levels following repeated MDMA *(experiment 4.1)*. Consistent to the results from *experiment 4.1*, the present study also showed that "binge-type" repeated administration of low dose MDMA (3 x 6 mg/kg) caused long-term impairment of novel object discrimination.

The effect of repeated MDMA administration (3 x 6 mg/kg) on body temperature in the group housed rats was similar to that seen in the singly housed rats shown in chapter 3, using radiotelemetry to measure body

temperature, as decreased body temperature was found after the first injection of MDMA (3 x 6 mg/kg) and then body temperature continuously increased following the second and the third injections. However the magnitude of body temperature increase is different as in the present study, using a rectal probe thermometer, MDMA produced a small increase of body temperature after the second and third injections (+0.4 °C using a rectal probe compared to +0.9 °C using radiotelemetry after the last injection compared to saline controls) and the increase was not significant compared to saline controls. This is possibly due to the lower sensitivity of a rectal probe compared to radiotelemetry. In addition, using a rectal probe, both saline and MDMA-treated rats showed a marked decrease of body temperature during the treatment period and it is noticeable that the first rectal temperature measured 1 h before treatment was as high as 39 °C. Animal restraint stress might affect the results of changes in body temperature when using a rectal probe. Together with the results from chapter 3 it is likely that MDMA-induced changes in body temperature depends mainly on ambient temperature rather than the housing conditions.

The influence of MDMA-induced acute hyperthermia on subsequent 5-HT neurotoxicity is unclear (Baumann et al., 2008a, Green et al., 2004, Malberg and Seiden, 1998, Sanchez et al., 2004). Many studies showed that preventing MDMA-induced hyperthermia using several compounds such as α-methyl-*p*-tyrosine, the tyrosine hydroxylase inhibitor, haloperidol, the dopamine receptor antagonist, and MDL 11939, the 5-HT_2 receptor antagonist, can protect or attenuate MDMA-induced neurotoxicity (Broening et al., 1995, Colado et al., 1999c, Hewitt and Green, 1994, Malberg and Seiden, 1998). In contrast many

studies demonstrated that although rat body temperature was kept normal at the time of MDMA administration, MDMA can produce 5-HT neurotoxicity (Farfel and Seiden, 1995, Marston et al., 1999, McGregor et al., 2003). O'Shea et al (1998) also reported MDMA-induced hyperthermia without subsequent 5-HT depletion. Similarly in the present study MDMA administration in the group housed rats produced a small but not significant increase in body temperature but long-term 5-HT depletion was found in the hippocampus. Comparing to *experiment 4.1* in which MDMA treatment in singly housed rats produced a similar increase of body temperature with no long-term effect on brain 5-HT levels, it is indicated that hyperthermia is not an essential factor in MDMA-induced 5-HT neurotoxicity.

In summary, both experiments in this chapter showed long-term disruption of novel object discrimination after "binge-type" repeated MDMA administration indicating risk of long-term effect on working as well as recognition memory impairments in "binge" MDMA users. This memory impairment following repeated MDMA administration however did not depend on 5-HT and dopamine function. In addition the present study provides evidence to show a lack of effect of aggregation on MDMA-induced changes in body temperature.

CHAPTER 5

INFLUENCE OF BRAIN TYROSINE AVAILABILITY ON MDMA-INDUCED 5-HT NEUROTOXICITY

5.1. Introduction

The precise mechanism underlying MDMA-induced 5-HT neurotoxicity is unknown; dopamine has been suggested to be involved. Sprague et al (1998) proposed that dopamine released following MDMA administration can be transported into 5-HT nerve terminals where oxidation of excessive dopamine produces free radicals which in turn induced the long-term damage to 5-HT terminals. Although administration of the tyrosine hydroxylase inhibitor, α-methyl-*p*-tyrosine, the dopamine depleting agent, reserpine, the dopamine uptake inhibitors, GBR 12909 (Stone et al., 1988) and mazindol (Shankaran et al., 1999), or the dopamine receptor antagonist, haloperidol (Hewitt and Green, 1994, Schmidt et al., 1990), attenuate the MDMA-induced loss of 5-HT which appears to support the proposal, it is likely that all of these compounds do so by decreasing body temperature rather than because of a specific effect on dopamine function (Colado et al., 1999b, Hekmatpanah et al., 1989, Yuan et al., 2002). Furthermore recent studies which examined the effect of depleting the cerebral dopamine content with α-methyl-*p*-tyrosine while keeping the animals normothermic (Yuan et al., 2002) or enhancing dopamine content by administering L-DOPA (Colado et al., 1999b) both failed to support a major role of dopamine in MDMA-induced neurotoxicity.

There are questions as to how dopamine can be involved in the MDMA-induced damage 5-HT neurons in several brain areas such as the hippocampus which are sparsely innervated by dopaminergic neurons. Recently Breier et al (2006) showed that MDMA increased the extracellular tyrosine concentration

in the striatum and hippocampus and demonstrated that this tyrosine could be converted to DOPA and dopamine via a tyrosine hydroxylase-independent mechanism. They also suggested that this increased dopamine concentration was primarily responsible for MDMA-induced 5-HT neurotoxicity

The aim of the experiment in this chapter was to determine whether acute brain tyrosine depletion would influence the MDMA-induced immediate 5-HT depletion and/or the long-term 5-HT neurotoxicity. To reduce tyrosine availability in the brain and consequently alter dopamine synthesis and release, rats were administered a tyrosine- and phenylalanine-free amino acid mixture. As tyrosine is competitively transported into the brain and nerve terminals by the large neutral amino acid transporter (Fernstrom and Wurtmanm, 1972, Grahame-Smith and Parfitt, 1970), administration of a tyrosine- and phenylalanine-free amino acid mixture reduces brain tyrosine levels by up to 70% within 2 hours (Biggio et al., 1976, Fernstrom and Fernstrom, 1995, McTavish et al., 1999). This experimental approach was therefore used to test the hypothesis that the brain tyrosine availability is involved in MDMA-induced 5-HT neurotoxicity.

5.2. Acute effects of tyrosine depletion on brain tyrosine, dopamine, 5-HT and their metabolite levels

5.2.1 Materials and methods

Animals

Male Dark Agouti rats weighing 165-200 g (Harlan-Olac, Bicester, UK) were housed on a 12h light/dark cycle (lights on at 07.00 h) and given food and water *ad libitum*. Room temperature (21 ± 2 °C) and humidity (45-65%) were kept constant. All experiments were performed in accordance with the UK Animals (Scientific Procedures) Act, 1986 under project license 40/2715 and approval of the local ethical committee. The experimenter was blind to the treatment given in all parts of the study.

Dark Agouti rats were used in the present study as this strain of rats is more sensitive to the acute and long-term effects of MDMA than other rat strains. It was shown that Dark Agouti rats required a single dose of MDMA (10-15 mg/kg) to produce 30-50% loss of cerebral 5-HT content (Colado et al., 1995, 1999b, O'Shea et al., 1998) while several doses of MDMA (10-20 mg/kg) were required to produce similar loss in Lister hooded rats (Colado et al., 1993, Sumnall et al., 2004).

Drugs and reagents

The tyrosine- (and phenylalanine-) free amino acid mixture was prepared as previously described by McTavish et al (1999) and contained methionine (133 mg), threonine (266 mg), tryptophan (67 mg), lysine (465 mg), isoleucine

methyl ester (399 mg), leucine methyl ester (599 mg) and valine methyl ester (465 mg) dissolved in 12 ml distilled water adjusted to pH 7.4 with 0.1 M NaOH and administered in a volume of 5 ml/kg i.p.

(±)-MDMA hydrochloride (Sigma) was dissolved in 0.154 M saline at 12.5 mg/kg (calculated as base) and administered in a volume of 1 ml/kg i.p.

Experimental design

Rats were divided into 4 groups (n = 5 per group) each housed by group. Two groups of rats received the tyrosine-free amino acid mixture (5 ml/kg i.p. equivalent to 1 g/kg twice 1 h apart) and other two groups received saline (5 ml/kg i.p. twice 1 h apart). One of the amino acid mixture-treated groups and one of the saline-treated groups received MDMA (12.5 mg/kg i.p.) 55 min after the first injection of the amino acid mixture (or saline) while the other two groups were given saline (1 ml/kg i.p.) in place of MDMA. Rats were killed by concussion and decapitated 2 h after MDMA administration. The hippocampus, striatum and frontal cortex were rapidly dissected on a cool tray at 4 °C, snap frozen in liquid nitrogen and maintained at -80 °C prior to the analysis of tyrosine, 5-HT, dopamine and their metabolites (experimental design summarised in Figure 5.1. Measurement of 5-HT, dopamine and their metabolites was described in chapter 2.

Measurement of tyrosine using HPLC-ECD

For the measurement of tyrosine, high-performance liquid chromatography (HPLC) with pre-column derivatisation coupled with electrochemical detection

(ECD) was utilized as the method has high sensitivity and specificity. The HPLC-ECD method was modified from Bongiovanni et al (2001). The reaction of *o*-phthalaldehyde (OPA) with tyrosine in the presence of sulfite was used to produce *N*-alkyl-1-isoindole sulphonates which were detected using ECD. The derivatisation reaction is presented in Figure 5.2A. The optimum pH for the reaction is between 9 and 10.5 (Jacobs, 1987) which in this experiment was achieved using borate buffer pH 10.4 (see *Derivatisation*).

The HPLC system consisted of a SphereClone column (4.6 x 100 mm, ODS 2, 3-µm particles, Phenomenex, Macclesfield, GB), a PU-980 pump (Jasco, Essex, UK) and an Antec Cu-04 electrochemical controller and Antec VT-03 electrochemical detector (Antec Leyden, Zoeterwoude, The Netherlands) fitted with a glassy carbon electrode set at +0.75V vs Ag/AgCl. The mobile phase consisting of 0.133 M disodium hydrogen phosphate (Na_2HPO_4), 0.15 mM EDTA and 20% v/v methanol adjusted to pH 6.8 with *o*-phosphoric acid was pumped at 0.5 ml/min flow rate. During sample injection the system was run open-circuit but mobile phase was recycled overnight or when not in use.

Tyrosine standard

Tyrosine stock solution was prepared by dissolving 10 mg of tyrosine (calculated as base) into 9.5 ml of diluents (water-methanol, 75:50 v/v) and 0.5 ml 30% NaOH. The 1 mg/ml of tyrosine stock solution was diluted with 0.1 M perchloric acid containing 1 µg/ml norvaline (as internal standard) to make working standards in µg/ml.

Derivatising agent

The derivatising reagent consisted of 10 mg *o*-phthalaldehyde, 30 mg sodium sulphite, 0.25 ml methanol and 0.25 ml H_2O diluted to 5.0 ml with sodium borate buffer pH 10.4 (0.4 M boric acid adjusted to pH 10.4 with 6 M NaOH). The derivatising reagent was prepared once weekly and stored at room temperature in an amber bottle.

Sample preparation

One ml of ice-cold 0.1 M perchloric acid containing 1 μg/ml norvaline (as internal standard) was added to each brain region in an Eppendorf tube and samples sonicated for 30s and centrifuged at 16000 g for 4 min at 4 °C (Harrier 18/80 refrigerated, MSE). The supernatants were stored at -80 °C until analysis.

Derivatisation

To detect tyrosine, 100 μl of sample or working standard (1 μg/ml) was routinely reacted for 5 min at the room temperature with 100 μl of derivatising agent and 100 μl of sodium borate buffer pH 10.4 (to buffer the perchloric acid used for the extraction). After 5 min the reaction samples or standards were made up to 1 ml with mobile phase before injection (20-μl) into the HPLC system. The minimum level of detection was 0.5 ng injected onto the column.

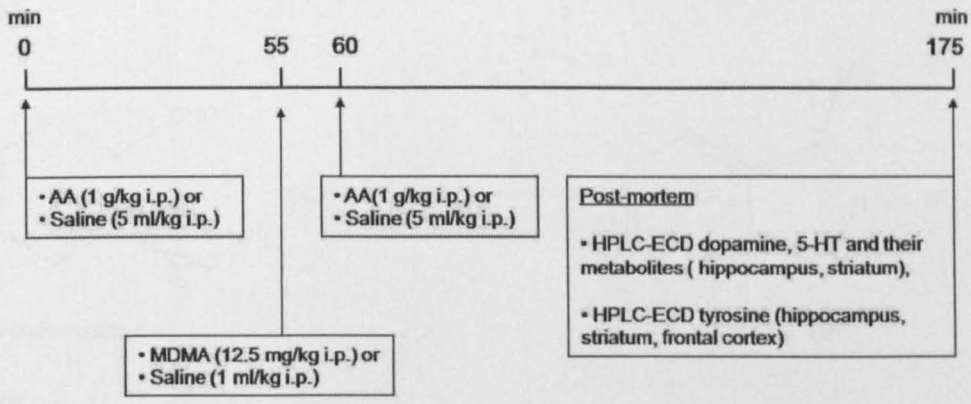
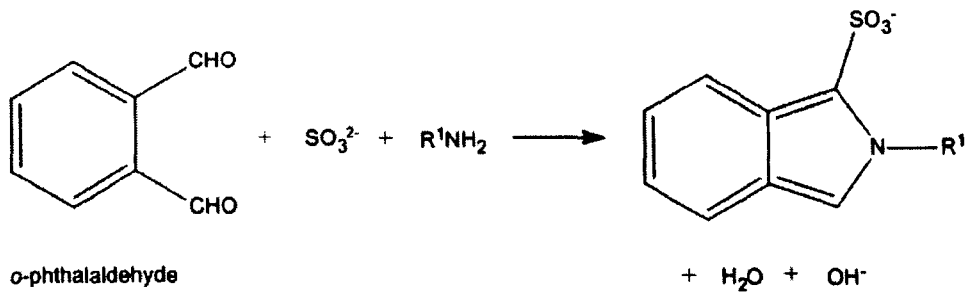


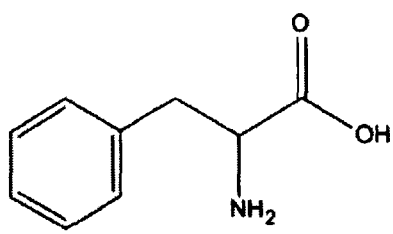
Figure 5.1 Diagrammatic representation of experimental protocol for determining acute effects of tyrosine depletion and MDMA on tyrosine, 5-HT, dopamine and their metabolite levels in specific brain regions

(A)



o-phthalaldehyde

(B)



Tyrosine

Figure 5.2 (A) Reaction scheme to illustrate the derivatisation reaction between *o*-phthalaldehyde and primary alkyl amine (R^1NH_2) in the presence of sulfite ions (SO_3^{2-}) producing *N*-alkyl-1-isoindole sulphonates. (B) The primary alkyl amine (R^1NH_2) reacted to *o*-phthalaldehyde in the present experiment is tyrosine.

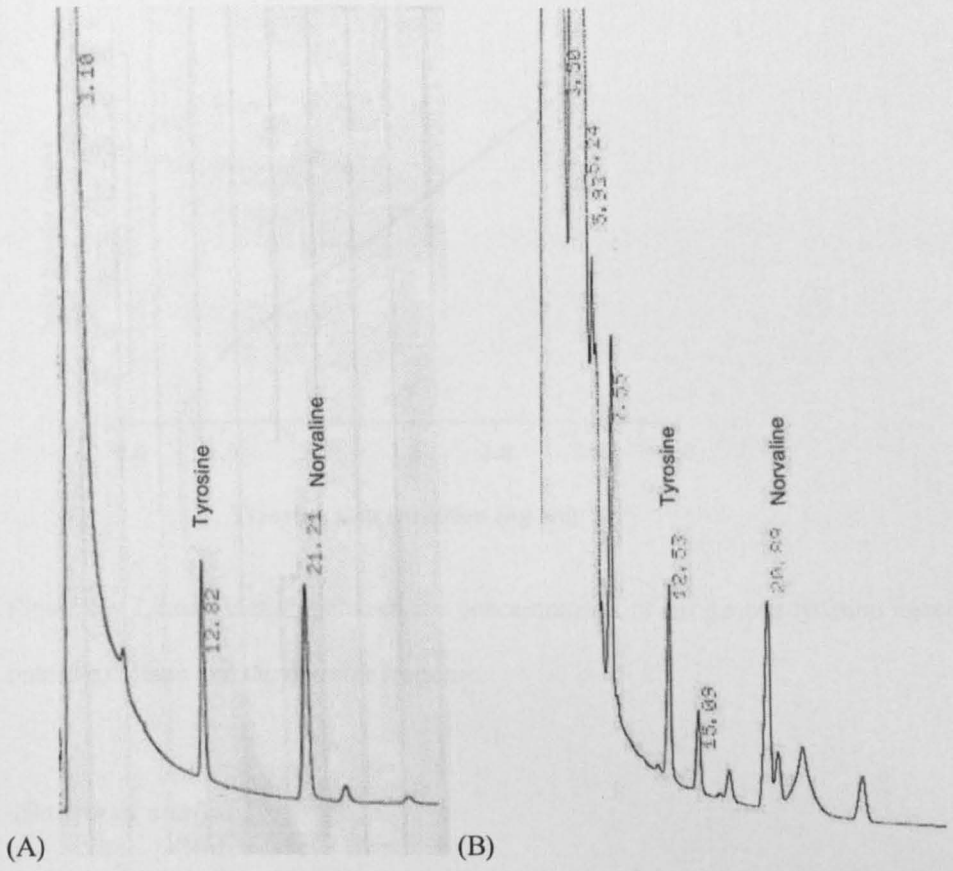


Figure 5.3 An example of the chromatographic separation of **A:** tyrosine and norvaline mixed standard (1 μg/ml each) and **B:** tyrosine in the striatum samples in percholic acid containing 1 μg/ml norvaline (as internal standard) using mobile phase 0.133 M Na_2HPO_4, 0.15 mM EDTA, 20 % v/v methanol pH 6.8.

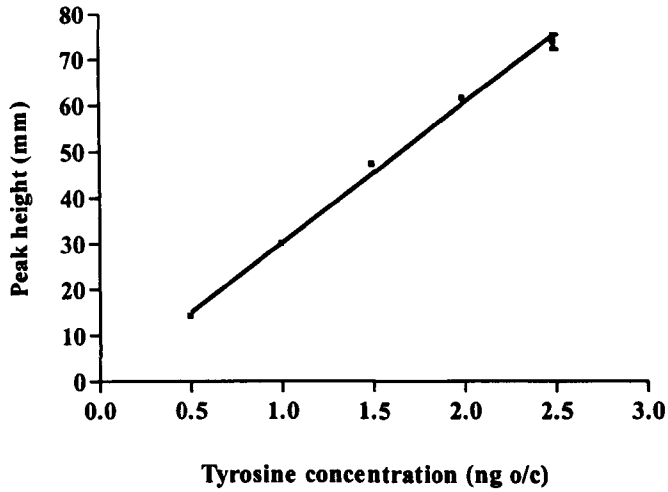


Figure 5.4 Linear relation between the concentrations of exogenous tyrosine injected onto the column and the detector response.

Statistical analysis

Statistical analysis of brain amines and tyrosine levels was performed using two-way ANOVA, with MDMA and tyrosine-free amino acid mixture as main factors followed by Bonferroni *post hoc* test if required. $P < 0.05$ was considered to be statistically significant.

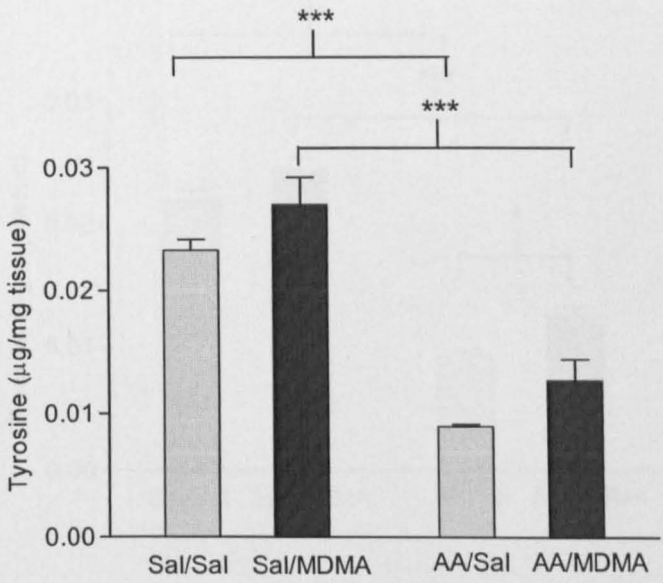
5.2.2 Results

(a) Acute effect of the tyrosine-free amino acid mixture and MDMA on tyrosine levels in the hippocampus, striatum and frontal cortex

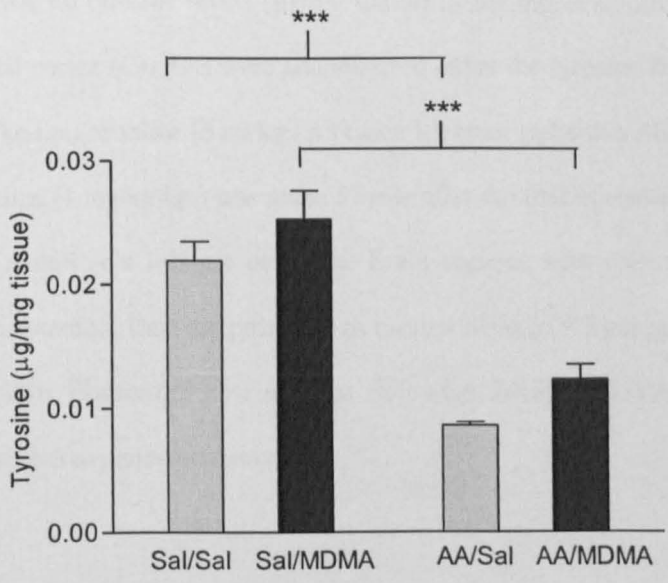
Tyrosine levels in the hippocampus, striatum and frontal cortex were measured 2 h after MDMA administration (Figure 5.5). Two-way ANOVA revealed a main effect of the tyrosine-free amino acid mixture on tyrosine levels in the hippocampus ($F(1,16) = 92.08$, $p < 0.0001$), striatum ($F(1,16) = 76.92$, $p < 0.0001$) and frontal cortex ($F(1,16) = 220.6$, $p < 0.0001$). The tyrosine-free amino acid mixture (AA/Sal) decreased hippocampal tyrosine levels by 62% compared with saline controls (Sal/Sal) ($p < 0.001$) while combined tyrosine-free amino acid mixture and MDMA (AA/MDMA) decreased hippocampal tyrosine levels by 53% compared with MDMA given alone (Sal/MDMA) ($p < 0.001$) (Figure 5.5A). Tyrosine levels were similarly reduced in the striatum by the tyrosine-free amino acid mixture without MDMA (AA/Sal) (61%; $p < 0.001$) compared with saline controls (Sal/Sal) and the tyrosine-free amino acid mixture given with MDMA (AA/MDMA) (51%; $p < 0.001$) compared with MDMA given alone group (AA/MDMA) (Figure 5.5B). In the frontal cortex, tyrosine levels decreased by the tyrosine-free amino acid mixture alone (AA/Sal) by 58% compared to saline controls (Sal/Sal) while combining the tyrosine-free amino acid mixture with MDMA (AA/MDMA) decreased tyrosine levels by 49% compared with MDMA alone (Sal/MDMA) ($p < 0.001$) (Figure 5.5C).

Two-way ANOVA also revealed that MDMA has an overall effect on tyrosine levels in the hippocampus, striatum and frontal cortex ($F(1,16) = 6.26$, $p = 0.0236$; $F(1,16) = 5.56$, $p = 0.0314$ and $F(1,16) = 12.13$, $p = 0.0031$ respectively). Irrespective of whether rats received the tyrosine-free amino acid mixture or saline, MDMA caused a similar magnitude increase in CNS tyrosine content. Consequently, compared with saline controls (Sal/Sal) MDMA (Sal/MDMA) increased tyrosine levels by 16% in the hippocampus, 15% in striatum and 11% in frontal cortex and in the tyrosine-free amino acid mixture group administered MDMA (AA/MDMA) it was 42% higher in the hippocampus, 43% higher in the striatum and 36% higher in the frontal cortex compared with the tyrosine-free amino acid given alone (AA/Sal).

(A) Hippocampus



(B) Striatum



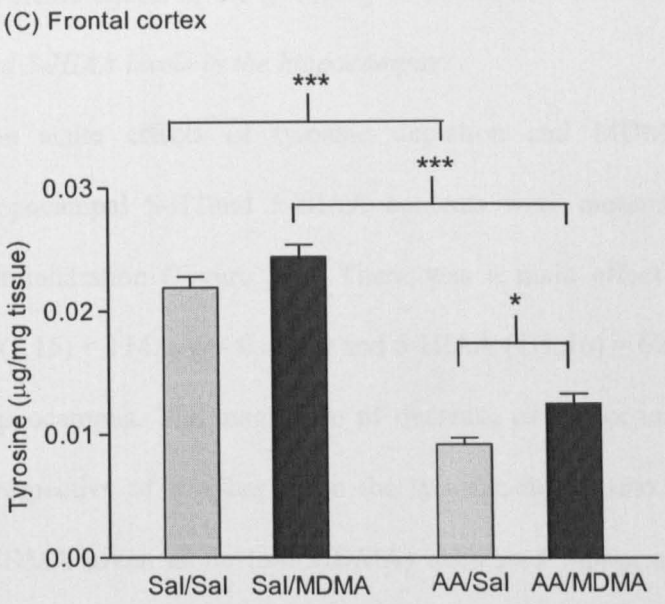


Figure 5.5 Effect of the tyrosine-free amino acid mixture given with or without MDMA on tyrosine levels (µg/mg tissue) in the hippocampus (A), striatum (B) and frontal cortex (C). Rats were administered either the tyrosine-free amino acid mixture (1 g/kg i.p.) or saline (5 ml/kg i.p.) twice 1 h apart and either MDMA (12.5 mg/kg i.p.) or saline (1 mg/kg i.p.) was given 55 min after the first injection of either the tyrosine-free amino acid mixture or saline. Brain regions were collected 2 h after MDMA administration. Data are presented as mean ± SEM (n = 5 per group) * $p < 0.05$; *** $p < 0.0001$ Bonferroni *post hoc* test following 2-way ANOVA (Sal = saline; AA = tyrosine-free amino acid mixture)

(b) Acute effects of the tyrosine-free amino acid mixture and MDMA on 5-HT and 5-HIAA levels in the hippocampus

The acute effects of tyrosine depletion and MDMA administration on hippocampal 5-HTand 5-HIAA contents were measured 2 h after MDMA administration (Figure 5.6). There was a main effect of MDMA on 5-HT ($F(1,15) = 114.6$, $p < 0.0001$) and 5-HIAA ($F(1,16) = 62.91$, $p < 0.0001$) in the hippocampus. The magnitude of decrease of hippocampal 5-HT was similar irrespective of whether given the tyrosine-free amino acid or not, such that MDMA given alone (Sal/MDMA) decreased hippocampal 5-HT (68%; $p < 0.001$) compared with saline-injected controls (Sal/Sal) (Figure 5.6A). Pretreatment with the tyrosine-free amino acid mixture (AA/Sal) did not alter 5-HT levels and rats given MDMA with the tyrosine-free amino acid showed a 5-HT depletion which was similarly reduced by 68% ($p < 0.001$) compared with the tyrosine-free amino acid mixture given alone (AA/Sal) (Figure 5.6A). MDMA alone (Sal/MDMA) also decreased 5-HIAA in the hippocampus by 38% ($p < 0.001$) compared to saline controls while combining MDMA with the tyrosine-free amino acid (AA/MDMA) decreased 5-HIAA by 51% compared with the tyrosine-free amino acid alone (AA/Sal) ($p < 0.001$) (Figure 5.6B).

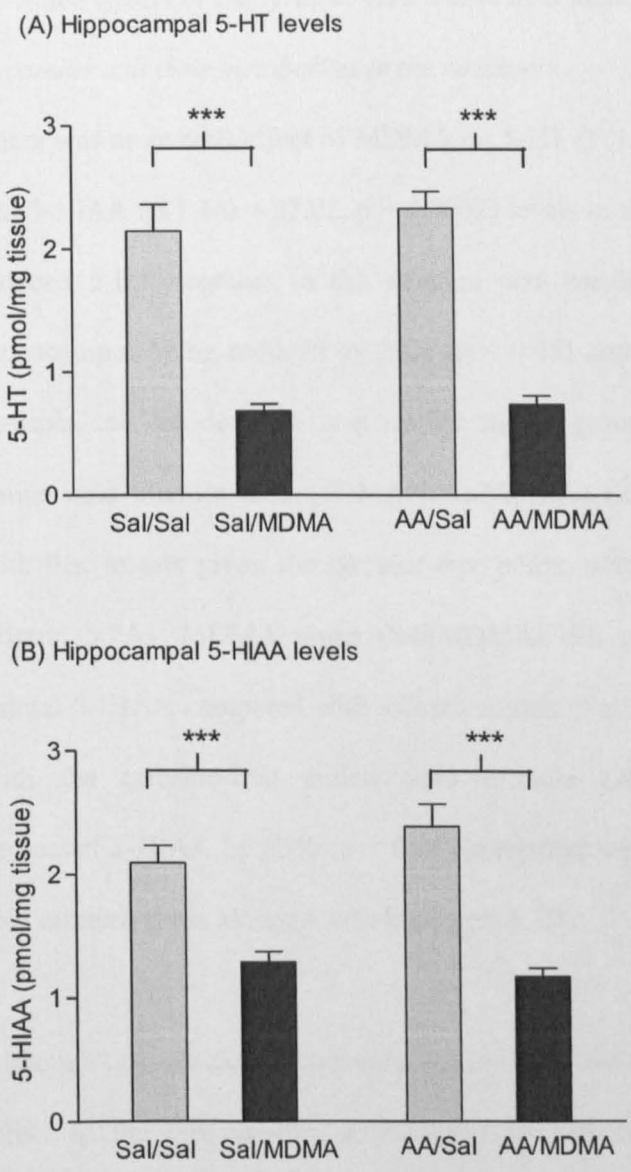


Figure 5.6 Lack of acute effect of tyrosine depletion on 5-HT (A) and 5-HIAA (B) levels in the hippocampus 2 h after MDMA administration. Rats were treated with either tyrosine-free amino acid mixture (1 g/kg i.p.) or saline (5 ml/kg i.p.) twice 1 h apart and then received either MDMA (12.5 mg/kg i.p.) or saline (1 mg/kg i.p.) 55 min after the first injection of the amino acid mixture or saline. Brain regions were collected 2 h after MDMA administration. Data are presented as mean ± SEM (n = 5 per group). *** $p < 0.001$ Bonferroni *post hoc* test following 2-way ANOVA (Sal = saline; AA = tyrosine-free amino acid mixture).

(c) Acute effects of the tyrosine-free amino acid mixture and MDMA on 5-HT, dopamine and their metabolites in the striatum

There was an overall effect of MDMA on 5-HT ($F(1,16) = 17.43$, $p = 0.0007$) and 5-HIAA ($F(1,16) = 23.02$, $p = 0.0002$) levels in the striatum. The MDMA-induced 5-HT decrease in the striatum was smaller than that seen in the hippocampus being reduced by 29% ($p < 0.05$) compared with that in saline controls and the decrease was similar in the group given the tyrosine-free amino acid mixture with MDMA (AA/MDMA) (25%, $p < 0.05$) compared with that in rats given the tyrosine-free amino acid mixture alone (AA/Sal) (Figure 5.7A). MDMA alone (Sal/MDMA) did not significantly decrease striatal 5-HIAA compared with saline controls (Sal/Sal) while giving MDMA with the tyrosine-free amino acid mixture (AA/MDMA) significantly decreased 5-HIAA by 25% ($p < 0.01$) compared with the tyrosine-free amino acid mixture given alone (AA/Sal) (Figure 5.7B).

Although tyrosine depletion had no effect on striatal 5-HT, there was an overall effect of the tyrosine-free amino acid mixture on striatal 5-HIAA levels ($F(1,16) = 6.49$, $p = 0.021$), such that giving the tyrosine-free amino acid mixture alone (AA/Sal) significantly increased 5-HIAA by 19% ($p < 0.05$) (Figure 5.7B).

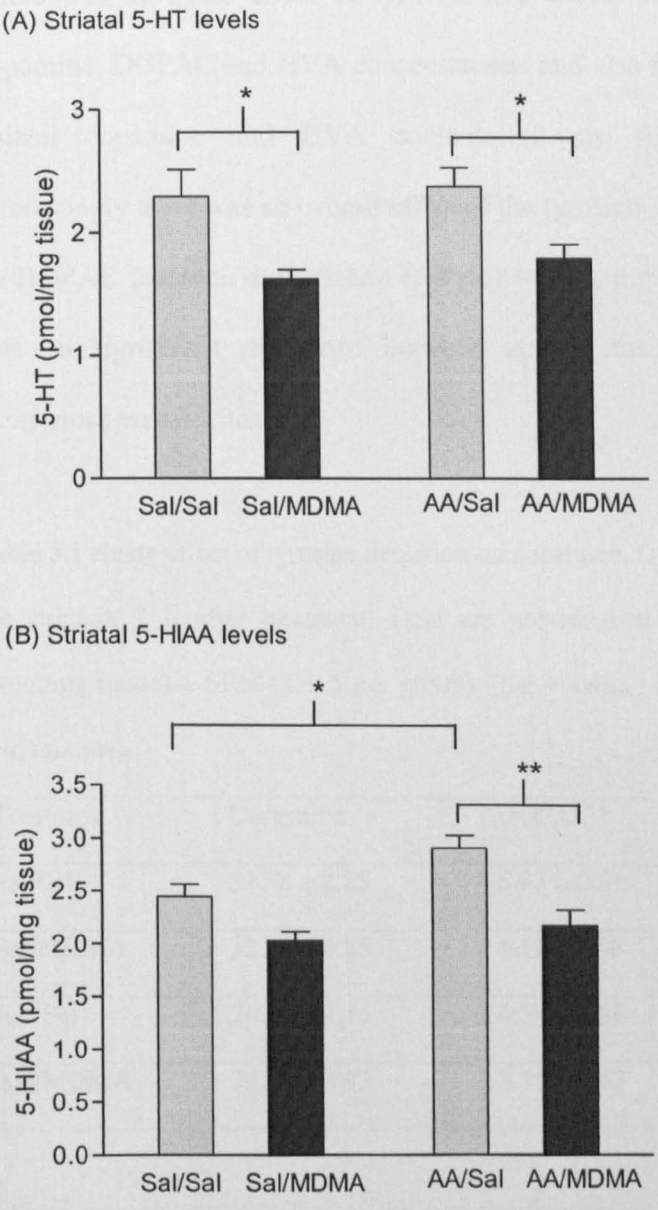


Figure 5.7 Acute effect of tyrosine depletion on 5-HT (A) and 5-HIAA (B) levels in the striatum 2 h after MDMA administration. Rats were treated with either tyrosine-free amino acid mixture (1 g/kg i.p.) or saline (5 ml/kg i.p.) twice 1 h apart and then received either MDMA (12.5 mg/kg i.p.) or saline (1 mg/kg i.p.) 55 min after the first injection of the amino acid mixture or saline. Brain regions were collected 2 h after MDMA administration. Data are presented as mean ± SEM (n = 5 per group) * $p < 0.05$; ** $p < 0.01$ Bonferroni *post hoc* test following 2-way ANOVA (Sal = saline; AA = tyrosine-free amino acid mixture)

There was no acute effect of tyrosine-free amino acid mixture on striatal dopamine, DOPAC and HVA concentrations and also no effect of MDMA on striatal dopamine and HVA contents (2-way ANOVA) (Table 5.1). Interestingly there was an overall effect of the tyrosine-free amino acid mixture on DOPAC levels in the striatum ($F(1,16) = 4.75$, $p = 0.045$). However there was no significant difference between any of the groups following the Bonferroni *post hoc* test.

Table 5.1 Acute effect of tyrosine depletion on dopamine, DOPAC and HVA levels in the striatum 2 h after treatment. Data are presented as means of concentration (pmol/mg tissue) ± SEM (n = 5 per group). (Sal = saline; AA = tyrosine-free amino acid mixture)

Treatment	Dopamine	DOPAC *	HVA
Sal/Sal	32.78 ± 2.85	5.83 ± 0.64	5.22 ± 0.38
Sal/MDMA	32.66 ± 4.85	4.13 ± 0.38	4.96 ± 0.68
AA/Sal	26.56 ± 4.16	4.54 ± 0.66	4.50 ± 0.94
AA/MDMA	38.67 ± 3.93	3.77 ± 0.53	5.38 ± 0.38

* There was an overall effect of the tyrosine-free amino acid mixture on DOPAC levels in the striatum ($F(1,16) = 4.75$, $p = 0.045$).

5.3. Effects of tyrosine depletion on MDMA-induced 5-HT neurotoxicity

5.3.1 Materials and methods

Animals

Male Dark Agouti rats weighing 165-200 g (Harlan-Olac, Bicester, UK) were housed on a 12h light/dark cycle (lights on at 07.00 h) and given food and water *ad libitum*. Room temperature (21 ± 2 °C) and humidity (45-65%) were kept constant. All experiments were performed in accordance with the UK Animals (Scientific Procedures) Act, 1986 under project license 40/2715 and approval of the local ethical committee. The experimenter was blind to the treatment given in all parts of the study.

Experimental procedure

A separate set of Dark Agouti rats were divided into 4 groups (n = 6 per group) and given one of the four treatments previously described in *5.2.1*. On the day of treatment, the rectal temperature of all rats was measured in lightly restrained rats using a digital thermocouple probe thermometer (Portec Instrumentation Ltd, P9005), 30 min after the first injection of the amino acid mixture or saline and immediately before the MDMA or saline injection, and again at 30, 60, 120 and 180 min after the second injection of the amino acid mixture or saline. The room temperature was kept constant at 21-23 °C. Rats were killed 14 days later and selected brain regions (hippocampus, striatum and frontal cortex) were dissected on a cool tray, snap frozen in liquid nitrogen and stored at - 80 °C until subsequent neurochemical analysis.

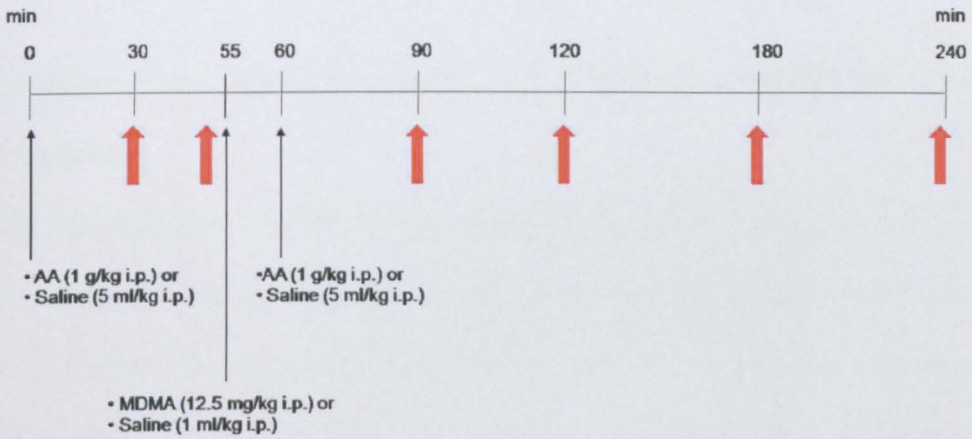


Figure 5.8 Treatment (black arrows) and rectal temperature measurement (red arrows) time on the treatment day. Either the tyrosine-free amino acid mixture (1 g/kg i.p.) or saline (5 ml/kg i.p.) was given twice 1 h apart (t = 0 and t = 60) and either MDMA (12.5 mg/kg i.p.) or saline (1 ml/kg i.p.) was given at t = 55. Rectal temperature was measured at the time indicated by red arrows.

Statistical analysis

Statistical analysis of 5-HT, dopamine and their metabolite levels was performed using two-way ANOVA, with MDMA and tyrosine-free amino acid mixture as main factors followed by Bonferroni *post hoc* test if required. Statistical analysis of rectal temperature was performed using two-way ANOVA, with time and treatment as main factors followed by Bonferroni *post hoc* test if required $P < 0.05$ was considered to be statistically significant.

5.3.2 Results

(a) Effect of the tyrosine-free amino acid mixture and/or MDMA on rectal temperature

On the treatment day the temporal changes in rectal temperature were measured after the first injection of the tyrosine-free amino acid mixture and for a further 180 min (see *Methods*). Two-way ANOVA revealed main effects of treatment ($F(3, 120) = 29.43$, $p < 0.0001$), time ($F(5,120) = 15.12$, $p < 0.0001$) and a treatment x time interaction ($F(15,120) = 6.84$, $p < 0.0001$) on rectal temperature. MDMA increased rectal temperature when given either with or without the tyrosine-free amino acid mixture while the tyrosine-free amino acid mixture alone had no effect on body temperature at any time point measured (Figure 5.9). It appears that MDMA given with the tyrosine-free amino acid mixture (AA/MDMA) increased body temperature more rapidly than when MDMA was given alone (Sal/MDMA) as the maximal hyperthermia in the tyrosine-free amino acid plus MDMA (AA/MDMA)-treated animals occurred at 30 min compared with 60 min after MDMA in saline plus MDMA (Sal/MDMA)-treated animals although the absolute peak rectal temperature was similar (+ 1.53 °C in AA/MDMA and + 1.70 °C in Sal/MDMA) (Figure 5.9). In addition, MDMA given alone caused a more prolonged increase of body temperature such that at 180 min after MDMA body temperature in the MDMA-treated animals was significantly greater than that in saline controls ($p < 0.01$) while the body temperature of MDMA plus tyrosine-free amino acid mixture-treated animals had returned to that observed in its appropriate control groups (Figure 5.9).

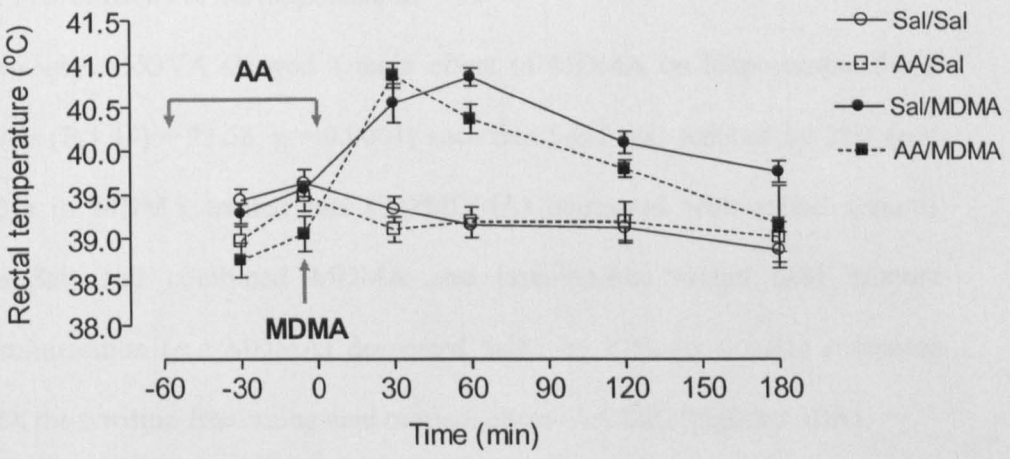


Figure 5.9 Effects of the tyrosine-free amino acid mixture given with or without MDMA on rectal temperature (°C, mean ± SEM, n = 6 per group). Rats were treated with either tyrosine-free amino acid mixture (1 g/kg i.p.) or saline (5 ml/kg i.p.) twice 1 h apart and given either MDMA (12.5 mg/kg i.p.) or saline (1 mg/kg i.p.) 55 min after the first injection of the amino acid mixture or saline. Rectal temperature was measured 30 min after the first injection of the amino acid mixture or saline and for further 180 min (see *Methods*).Two-way ANOVA revealed main effects of treatment ($F(3, 120) = 29.43$, $p < 0.0001$), time ($F(5,120) = 15.12$, $p < 0.0001$) and a treatment x time interaction ($F(15,120) = 6.84$, $p < 0.0001$) on rectal temperature. At 30 min after the first injection of the amino acid mixture, there was a significant difference in rectal temperature between AA/MDMA group and saline controls ($p < 0.05$). MDMA alone produced a significant increase in rectal temperature at 30 ($p < 0.001$), 60 ($p < 0.001$), 120 ($p < 0.01$) and 180 ($p < 0.01$) min compared with that in saline controls. In addition MDMA given with the tyrosine-free amino acid mixture showed a significantly different change in rectal temperature from controls at 30 ($p < 0.001$), 60 ($p < 0.001$) and 120 ($p < 0.05$) min after the second injection of tyrosine-free amino acid mixture.

(b) Long-term effects of tyrosine depletion and MDMA on MDMA-induced 5-HT neurotoxicity in the hippocampus

Two-way ANOVA showed a main effect of MDMA on hippocampal 5-HT levels ($F(1,19) = 23.58$, $p = 0.0001$) such that 5-HT was reduced by 28% ($p < 0.01$) in MDMA treated rats (Sal/MDMA) compared with saline controls (Sal/Sal) and combined MDMA and tyrosine-free amino acid mixture administration (AA/MDMA) decreased 5-HT by 27% ($p < 0.01$) compared with the tyrosine-free amino acid mixture alone (AA/Sal) (Figure 5.10A).

There were MDMA ($F(1,20) = 11.58$, $p = 0.0028$) and MDMA x AA interaction effects ($F(1,20) = 5.97$, $p = 0.024$) on 5-HIAA levels in the hippocampus. MDMA alone (Sal/MDMA) significantly decreased 5-HIAA levels in the hippocampus by 24% ($p < 0.01$) compared with saline controls (Sal/Sal) while pretreatment with tyrosine-free amino acid mixture showed no significant difference of 5-HIAA levels in the hippocampus when given either with or without MDMA (Figure 5.10B).

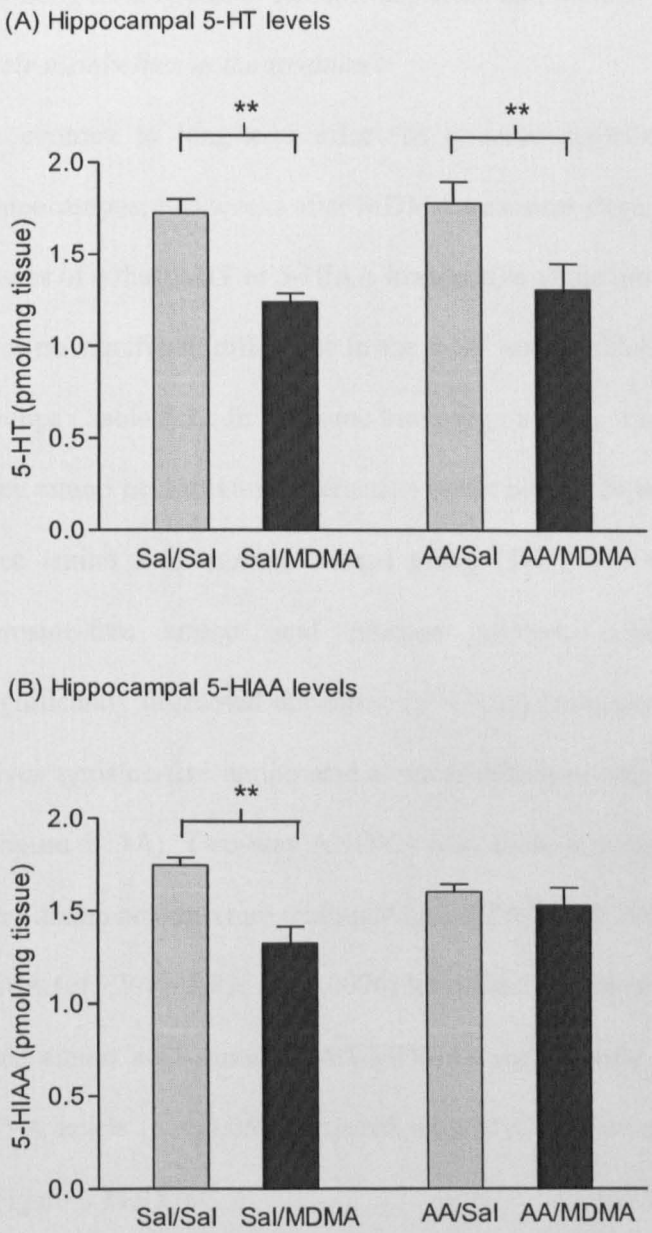


Figure 5.10 Long-term effect of tyrosine depletion on 5-HT (A) and 5-HIAA (B) levels in the hippocampus 2 weeks after MDMA administration. Rats were treated with either tyrosine-free amino acid mixture (1 g/kg i.p.) or saline (5 ml/kg i.p.) twice 1 h apart and then received either MDMA (12.5 mg/kg i.p.) or saline (1 mg/kg i.p.) 55 min after the first injection of the amino acid mixture or saline. Data are presented as mean ± SEM (n = 6 per group). ** $p < 0.01$ Bonferroni *post hoc* test following 2-way ANOVA (Sal = saline; AA = tyrosine-free amino acid mixture)

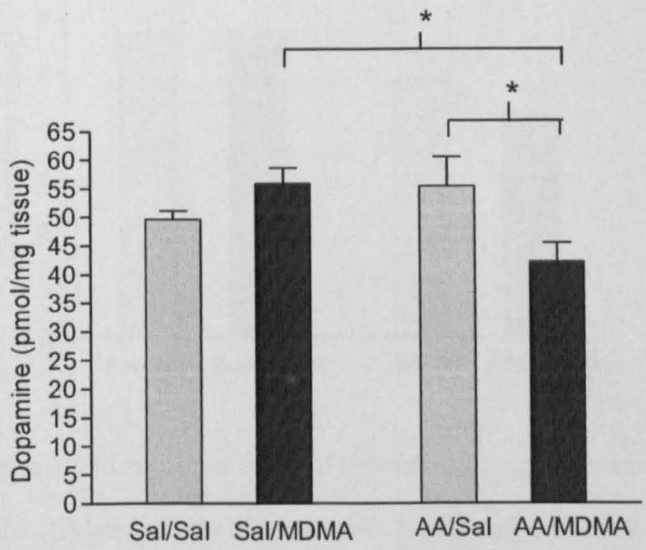
(c) Long-term effects of tyrosine depletion and MDMA on 5-HT, dopamine and their metabolites in the striatum

In contrast to long-term effect of tyrosine depletion and MDMA in the hippocampus, two weeks after MDMA treatment there were no clear long-term losses of either 5-HT or 5-HIAA irrespective of the drug combination and there was no significant difference in the 5-HT and 5-HIAA levels in any treatment groups (Table 5.2). In the same brain region there was an MDMA x tyrosine-free amino acid mixture interaction effect on the dopamine levels in tyrosine-free amino acid mixture treated group ($F(1,19) = 9.28$, $p = 0.0067$). The tyrosine-free amino acid mixture MDMA combination (AA/MDMA) significantly decreased dopamine ($p < 0.05$) compared with that in rats either given tyrosine-free amino acid alone (AA/Sal) or MDMA alone (Sal/MDMA) (Figure 5.11A). Two-way ANOVA also showed an overall effect of tyrosine-free amino acid mixture treatment on DOPAC ($F(1,20) = 8.49$; $p = 0.0086$) and HVA ($F(1,20) = 8.82$; $p = 0.0076$) levels in the striatum. MDMA with tyrosine-free amino acid mixture (AA/MDMA) significantly decreased DOPAC and HVA levels ($p < 0.05$) compared with MDMA treatment alone (Sal/MDMA) (Figure 5.11B,C).

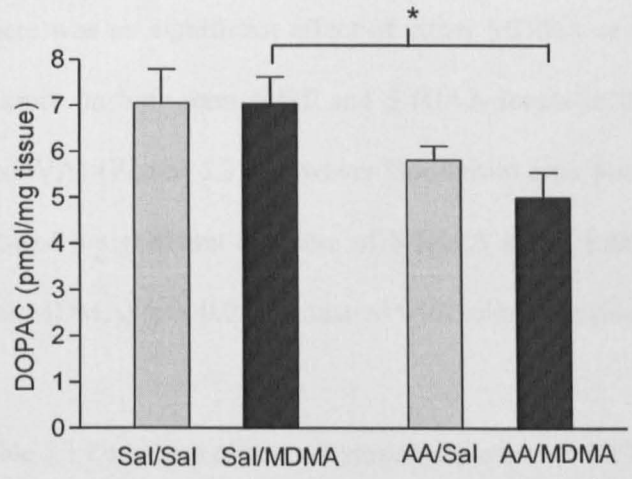
Table 5.2 Lack of long-term effect of tyrosine depletion and MDMA on 5-HT and 5-HIAA levels in the striatum 2 weeks after treatment. Data are presented as means of concentration (pmol/mg tissue) ± SEM (n = 6 per group).

Treatment	5-HT	5-HIAA
Sal/Sal	2.38 ± 0.16	2.32 ± 0.15
Sal/MDMA	2.26 ± 0.18	2.18 ± 0.14
AA/Sal	2.38 ± 0.07	2.18 ± 0.08
AA/MDMA	2.72 ± 0.25	2.41 ± 0.21

(A) Striatal dopamine levels



(B) Striatal DOPAC levels



(C) Striatal HVA levels

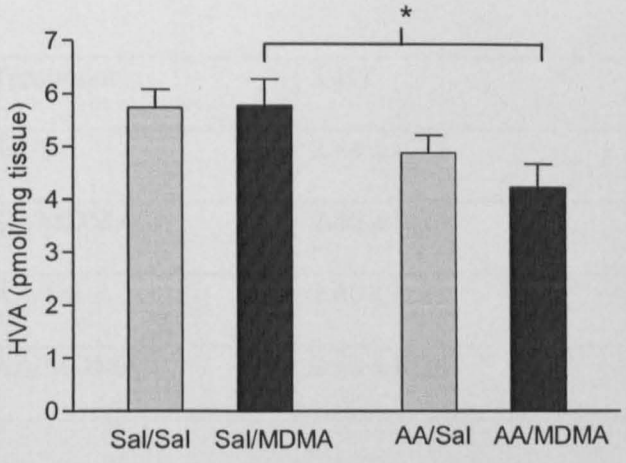


Figure 5.11 Long-term effect of tyrosine depletion on dopamine (A) DOPAC (B) and HVA (C) levels in the hippocampus 2 weeks after MDMA administration. Rats were treated with either tyrosine-free amino acid mixture (1 g/kg i.p.) or saline (5 ml/kg i.p.) twice 1 h apart and then received either MDMA (12.5 mg/kg i.p.) or saline (1 mg/kg i.p.) 55 min after the first injection of the amino acid mixture. Data are presented as mean ± SEM (n = 6 per group). * $p < 0.05$ Bonferroni *post hoc* test following 2-way ANOVA (Sal = saline; AA = tyrosine-free amino acid mixture).

(d) Long-term effects of tyrosine depletion and MDMA 5-HT and 5-HIAA levels in the frontal cortex

There was no significant effect of either MDMA or tyrosine-free amino acid mixture on long-term 5-HT and 5-HIAA levels in the frontal cortex (2-way ANOVA) (Figure 5.3). However Bonferroni *post hoc* test following ANOVA showed a significant decrease of 5-HIAA levels following MDMA treatment (Sal/MDMA) ($p < 0.05$) compared with saline controls (Sal/Sal).

Table 5.3 Long-term effects of tyrosine depletion on 5-HT and 5-HIAA levels in the frontal cortex 2 weeks after treatment. Data are presented as means of concentration (pmol/mg tissue) ± SEM (n = 6 per group).

Treatment	5-HT	5-HIAA
Sal/Sal	2.84 ± 0.27	1.36 ± 0.09
Sal/MDMA	2.40 ± 0.25	1.05 ± 0.08*
AA/Sal	2.60 ± 0.27	1.15 ± 0.09
AA/MDMA	2.48 ± 0.24	1.13 ± 0.07

* $P < 0.05$ compared with Sal/Sal (Bonferroni *post hoc* test following 2-way ANOVA)

5.4. Discussion

The experiments in this chapter investigated the contribution of tyrosine availability to the mechanisms underlying MDMA-induced brain 5-HT depletions. For this, brain tyrosine levels were depleted during MDMA administration by giving a tyrosine-free amino acid mixture. It was demonstrated that acute administration of the tyrosine-free amino acid mixture with and without MDMA produced a greater than 50% reduction of tyrosine in the hippocampus, striatum and frontal cortex 2 h after MDMA administration, confirming the finding of others (Biggio et al., 1976, Fernstrom and Fernstrom, 1995, McTavish et al., 1999). Despite acute tyrosine depletion in rats treated with a combination of the tyrosine-free amino acid mixture and MDMA, the tyrosine-free amino acid mixture did not attenuate MDMA-induced acute 5-HT and 5-HIAA loss in the hippocampus and striatum observed 2 h after MDMA administration.

Regardless of the effect of a tyrosine-free amino acid mixture, systemic MDMA administration has an overall effect on the brain tyrosine concentrations, such that MDMA alone produced a small increase in tyrosine levels (11-16 %) compared to saline controls and MDMA given with the tyrosine-free amino acid mixture increased the tyrosine levels (36-42 %) compared to the tyrosine-free amino acid mixture given alone (see *5.2.2a*). This observation is consistent with the finding of Breier et al (2006) in that the extracellular concentration of tyrosine increased following MDMA administration. However Breier et al (2006) administered 4 doses of MDMA

(10 mg/kg, 2 h apart) while a single 12.5 mg/kg dose of MDMA was given in the present study. The question arises as to the origin of the tyrosine. Breier et al. (2006) suggested a peripheral origin. Recently Goñi-Allo et al (2008) also reported that MDMA administration increased the serum tyrosine concentration which suggests that the rise in the brain may be the consequence of a preceding peripheral elevation.

At the doses given to Sprague Dawley rats, Breier et al. (2006) produced a decrease in 5-HT content of 60% in the hippocampus and 40% in the striatum 1 week after treatment. In the present study, using Dark Agouti rats and a dose of MDMA of 12.5 mg/kg i.p., there was a 28% decrease of 5-HT in the hippocampus. In contrast to the finding of Breier et al. (2006) the present study was unable to show that altering tissue free tyrosine influenced the long-term (2 weeks) effect of MDMA in the hippocampus in the present study. The 5-HT content in the striatum and frontal cortex however was not decreased following MDMA in this study. O'Shea et al (2006) have previously demonstrated that the striatum is less sensitive to the long-term effects of MDMA. Surprisingly there was no loss in 5-HT and 5-HIAA in the frontal cortex 2 weeks after MDMA.

Although microdialysis studies have shown that MDMA administration acutely increases dopamine release (Colado et al., 1999, Mechan et al., 2002a, Shankaran and Gudelsky, 1998), tissue dopamine levels in the striatum are generally reported to be either increased or unchanged (Colado and Green, 1994, Gough et al., 1991, Logan et al., 1988, Schmidt et al., 1987, Schmidt et

al., 1991, Yamamoto and Spanos, 1988) and in the present study no acute change of dopamine and its metabolites in the striatum was seen 2 h after MDMA. Acute tyrosine depletion also had no effect on striatal dopamine, DOPAC and HVA levels 2 h after the second injection of the tyrosine-free amino acid mixture, in agreement with previous studies showing that a tyrosine-free amino acid mixture failed to alter basal dopamine levels even though it attenuated amphetamine- and haloperidol-induced dopamine release (Jaskiw and Bongiovanni, 2004, McTavish et al., 1999). In addition the present study confirmed previous studies which have shown that MDMA alone does not produce long-term dopaminergic neurotoxicity in rats (Battaglia et al., 1987, Colado et al., 1997, Colado et al., 1999b). Administration of the tyrosine-free amino acid mixture together with MDMA however did produce a long-term decrease in striatal dopamine and its metabolites, an observation that needs further investigation and there is no explanation for this change at present.

What was altered by administration of the tyrosine-free amino acid mixture was the MDMA-induced hyperthermic response. While the peak temperature rise was comparable, the duration of the elevated rectal temperature was attenuated. Interestingly Goñi-Allo et al (2008) noted a modest enhancement of the MDMA-induced hyperthermic response when they pretreated rats with tyrosine. Together these observations suggest that increased cerebral tyrosine can enhance the MDMA-induced temperature response while tyrosine depletion can attenuate the hyperthermia duration. The mechanisms involved in this interaction are unclear at present. However the study of Mechan et al

(2002a) indicated that the hyperthermic effect of MDMA in animals kept at normal ambient room temperature was primarily the result of dopamine release and subsequent dopamine D_1 receptor activation. Since the tyrosine-free amino acid mixture has previously been shown to decrease amphetamine-induced dopamine release (McTavish et al., 1999) one might expect it to have the same effect on dopamine release induced by the substituted amphetamine, MDMA, thereby attenuating the temperature response. The modest attenuation of MDMA-induced hyperthermia seen in the current study therefore supports the notion that dopamine is associated with MDMA-induced hyperthermia.

In conclusion, this study shows that reducing brain tyrosine levels during MDMA administration does not have an indirect effect on either MDMA-induced immediate or long-term 5-HT loss. However the study does support the observation that MDMA increases the cerebral free tyrosine concentration and the proposal that dopamine is involved in MDMA-induced hyperthermia.

CHAPTER 6

GENERAL DISCUSSION

6.1 Introduction

The principle aim of this thesis was to determine the acute and long-term functional and pharmacological effects of single and 'binge type' repeated administration of low doses of MDMA in rats. The work focused on the effects of MDMA on memory and the possible association between 5-HT and these effects of MDMA.

The main findings of this thesis were as follows:

1. Single administration of a low dose of MDMA (3 mg/kg) produced an acute disruption of novel object discrimination indicating an acute impairment of recognition as well as working memory following MDMA (Chapter 2).

2. 'Binge type' repeated administration of low doses of MDMA increased locomotor activity (3 x 6 mg/kg), impaired thermoregulation (3 x 6 mg/kg) and increased 5-HT release in the hippocampus (3 x 3 mg/kg and 3 x 6 mg/kg) (Chapter 3).

3. The acute disruption of novel object discrimination following a single low dose MDMA administration (3 mg/kg) was associated with 5-HT release in the hippocampus (Chapter 2 and 3).

4. 'Binge type' repeated low dose MDMA administration produced long-term impairment of novel object discrimination indicating a long-term effect of

MDMA on recognition and working memory (Chapter 4). This long-term effect of MDMA is consistent with several studies in humans which report long-term recognition and working memory impairments in MDMA users. However the present study found no long-term effect of repeated low dose MDMA administration on 5-HT levels in the hippocampus, frontal cortex or striatum indicating 5-HT loss did not contribute to MDMA-induced memory impairment (Chapter 4).

5. MDMA increased brain tyrosine levels. However depletion of brain tyrosine, by giving a tyrosine-free amino acid mixture both before and after MDMA administration, did not protect against MDMA-induced immediate and long-term loss of 5-HT suggesting there was no involvement of tyrosine in MDMA-induced 5-HT neurotoxicity (Chapter 5). In addition the tyrosine-free amino acid mixture reduced onset and duration of MDMA-induced hyperthermia suggesting a possible association between dopamine and the hyperthermic response following MDMA.

Overall the results of the present study provide extensive evidence showing that low doses of MDMA can produce both acute and long-term memory impairments and these effects of MDMA do not correlate with changes in brain 5-HT. Three main topics will be discussed in this chapter. The first is how effectively data obtained from animal studies can be interpreted in terms of potential effects in humans. Secondly what is the evidence for 5-HT neurotoxicity following low doses MDMA administration and finally what are the possible mechanisms underlying MDMA-induced memory impairments.

6.2 The interpretation of animal data to humans

The study of functional and pharmacological effects of MDMA in this thesis attempted to relate the findings in rats to the possible consequences of MDMA use in humans therefore low doses and 'binge type' repeated administration of MDMA were applied. Green et al (2009) reviewed the pharmacokinetics of MDMA in rats and humans and produced MDMA dose-plasma concentration response curve for rats and humans and therefore suggested that "a fourfold higher dose is required in rats to produce a similar peak blood plasma exposure to that seen in human" (Green et al., 2009). Thus, doses of MDMA used in this thesis, 1, 3 and 6 mg/kg are equivalent to 0.25, 0.75 and 1.5 mg/kg in humans respectively which are in the range of doses used by humans (0.75-4 mg/kg) (Morgan, 2000). 'Binge' use of MDMA has been described recently as MDMA users reportedly taking on average 2-3 tablets during a single session (Parrott, 2005, Soar et al., 2006, Winstock et al., 2001). The experiments in this thesis used a dose regime of 3 injections of low dose MDMA every 2 h to imitate binge use in humans. Thus in terms of the doses and dosage regimen used in this thesis, every effort was made to use conditions in the rat that closely resemble use in humans.

The review by Green et al (2009) however did not detail other pharmacokinetic factors including route of administration, plasma protein binding and especially metabolism of MDMA. The differences in the MDMA metabolic pathways between rats and humans have been a concern as a limitation in the translation of animal data to humans as toxic metabolites rather than MDMA itself

produce neurotoxicity (Escobedo et al., 2005, Esteban et al., 2001). Although the metabolites of MDMA in rats and humans are qualitatively similar, the major metabolic pathways are different. In rats MDMA is mainly *N*-demethylated to MDA (3,4-methylenedioxyamphetamine) whereas MDMA is mainly metabolized to HHMA (3,4-dihydroxymethamphetamine) via *O*-demethylation in humans (De la Torre and Farre, 2004). Moreover human MDMA metabolism shows non-linear pharmacokinetics as MDMA acts as a CYP 2D6, an enzyme important in MDMA metabolism, inhibitor and consequently decreases MDMA metabolism (Delaforge et al., 1999, Farre et al., 2004) while there was no enzyme inhibition in rats (Capela et al., 2009, De la Torre and Farre, 2004). Therefore repeated administration of MDMA to rats might produce different effects from binge MDMA ingestion in humans. The influence of metabolism of MDMA on 'binge' use of MDMA needs further investigation.

6.3 Does low dose MDMA administration produce long-term 5-HT neurotoxicity?

Lack of evidence for MDMA-induced 5-HT neurotoxicity in rats following repeated low doses of MDMA administration (3 and 6 mg/kg i.p. 3 injections every 2 h) was presented in this thesis. The dosage regimen used in this thesis represents 'binge' use in a single occasion and the results indicate no long-term effect of this dosage regimen of MDMA on brain 5-HT in rats. There have been a few studies on long-term 5-HT neurotoxicity following repeated low doses MDMA administration. Studies by Baumann et al (2007, 2008b) using a

similar dosage regimen showed that the lower dose of MDMA (1.5 mg/kg i.p. 3 injections every 2 h) did not produce a long-term effect on 5-HT levels while the higher dose of MDMA (7.5 mg/kg i.p. 3 injections every 2 h) decreased 5-HT levels by ~50% in the frontal cortex, striatum, olfactory tubercle, nucleus accumbens and hypothalamus of the rats. A more intensive dosage regimen to rats (MDMA 5 mg/kg 4 injections for 2 days) decreased 5-HT in the prefrontal cortex, striatum, hippocampus and amygdala 20 weeks after treatment (McGregor et al., 2003). Similarly MDMA (4 mg/kg x 2 for 4 days) decreased 5-HT levels and [^{3}H]paroxetine binding to presynaptic 5-HT terminals 7 days after treatment in rat cortex, hippocampus and striatum (O'Shea et al., 1998). Finally a study by Kindlundh-Hogberg et al (2007), using 'binge' dosage regimen on several occasions (MDMA 3x 5 mg/kg every 3 h once a week for 4 weeks), showed a decrease of SERT density in the rat nucleus accumbens. Taken together, there is evidence for MDMA-induced 5-HT neurotoxicity following binge low doses of MDMA especially when using more intensive regimens or on repeated occasions. These results indicate that the frequency and duration of dosing influence the effects of MDMA on 5-HT neurotoxicity. The influence of the amounts of MDMA used on 5-HT neurotoxicity have also been reported in humans as there was a decrease of SERT binding in heavy MDMA users while normal SERT binding was found in moderate MDMA users (see reviews Cowan, 2007, de Win et al., 2004, Reneman et al., 2001a, 2001b). In summary it is likely that there is no long-term 5-HT neurotoxicity following low dose MDMA administration to rats and possibly in mild MDMA human users.

6.4 Mechanisms for memory impairments produced by MDMA

This thesis showed no contribution of 5-HT on the MDMA-produced long-term memory impairments following repeated low dose administration (binge). Although 5-HT appears to play important roles in learning and memory, several neurotransmitter systems have been implicated in the memory process including dopamine, acetylcholine and glutamate (see review Riedel et al., 2003, Vakalopoulos, 2006). Therefore changes in 5-HT might not be the only explanation for MDMA-induced memory impairments.

Recently adult neurogenesis in hippocampal dentate gyrus has been one of the factors linked with learning and memory (see review Suzuki and Clayton, 2000). Bruel-Jungerman et al (2005) showed that an increase in the number of adult-generated neurons in rat dentate gyrus contributed to improvement of long-term recognition memory using the novel object recognition task. Stimulants such as cocaine and methamphetamine were shown to reduce cell proliferation in the hippocampal dentate gyrus (Teuchert-Noodt et al., 2000, Yamaguchi et al., 2004, Dominguez-Escriba et al., 2006). Similarly Hernandez-Rabaza et al (2006) demonstrated that binge MDMA administration (5 mg/kg i.p. 8 injections every 6 h) has deleterious effects on adult neurogenesis by impairing the survival of newly generated neurons in the dentate gyrus while there was no effect on cell proliferation. In addition chronic MDMA administration (1.25, 5, 20 or 40 mg/kg p.o. for 30 days) to C57BL/6 mice decreased cell proliferation (Cho et al., 2007). Recently Cho et al (2008) showed a decrease of cell proliferation and survival in adult mouse

dentate gyrus following exposure to MDMA during early development. Therefore a decrease in adult neurogenesis following MDMA may possibly influence the memory impairments produced by MDMA. In addition 5-HT is one of the factors involved in the regulation of adult hippocampal neurogenesis (Brezun and Daszuta, 1999, Djavadian, 2004, Malberg et al., 2000). It is likely that an indirect effect of an MDMA-induced decrease in 5-HT is to decrease neurogenesis and thus might be implicated in the mechanism of memory impairments produced by MDMA.

6.5 Summary

MDMA use has increased dramatically and more intensive patterns of use such as binging have become general. MDMA is thought by some to be a safe drug (Nutt, 2009) and there are several studies showing no 5-HT neurotoxicity following mild or moderate use. In addition loss of 5-HT is reversed following long term abstinence in rats and humans. However the psychological consequences of MDMA use have been intensively reported especially the impairments of memory and learning abilities which persist in MDMA users. Although specific studies in humans are limited due to several factors including ethical issues concerning drug administration, history of drug use and polydrug use, studies in animals have demonstrated learning and memory impairments caused by MDMA. The translation of these preclinical data to humans is a problem due to issues raised by irrelevant dose administration and dosage regimens between animal and human studies. The results from this thesis provide strong evidence for acute and long-term memory impairments

caused by low dose MDMA administration in rats and that these effects may translate effectively to human conditions. Consistent with studies in humans, low dose MDMA administration appears to have no long-term effect on brain 5-HT in the rat indicating no contribution of 5-HT to MDMA-induced memory impairments observed with these low dose regimens. Therefore it is important to focus on factors other than 5-HT when studying the mechanism involved in MDMA-induced memory impairments for example to determine whether the memory impairment involves the attenuation of neurogenesis by MDMA. Further studies also need to address the effect of MDMA metabolism, which differs between rat and human, on the 'binge' effects of MDMA.

REFERENCES

ABLE, J. A., GUDELSKY, G. A., VORHEES, C. V. & WILLIAMS, M. T. (2006) 3,4-Methylenedioxymethamphetamine in adult rats produces deficits in path integration and spatial reference memory. *Biological Psychiatry*, 59, 1219-26.

ACQUAS, E., MARROCU, P., PISANU, A., CADONI, C., ZERNIG, G., SARIA, A. & DI, C. G. (2001) Intravenous administration of ecstasy (3,4-methylendioxymethamphetamine) enhances cortical and striatal acetylcholine release in vivo. *Eur. J. Pharmacol.*, 418, 207-211.

AGGLETON, J. P. & BROWN, M. W. (1999) Episodic memory, amnesia, and the hippocampal-anterior thalamic axis. Behav Brain Sci, 22, 425-44.

AGUIRRE, N., BARRIONUEVO, M., RAMIREZ, M. J., DEL, R. J. & LASHERAS, B. (1999) Alpha-lipoic acid prevents 3,4-methylenedioxy-methamphetamine (MDMA)-induced neurotoxicity. *Neuroreport*, 10, 3675-3680.

AL-SAHLI, W., AHMAD, H., KHERADMAND, F., CONNOLLY, C. & DOCHERTY, J. R. (2001) Effects of methylenedioxymethamphetamine on noradrenaline-evoked contractions of rat right ventricle and small mesenteric artery. *Eur. J. Pharmacol.*, 422, 169-174.

ALEX, K. D. & PEHEK, E. A. (2007) Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. *Pharmacology & Therapeutics*, 113, 296-320.

ALVES, E., SUMMAVIELLE, T., ALVES, C. J., GOMES-DA-SILVA, J., BARATA, J. C., FERNANDES, E., BASTOS, M. L., TAVARES, M. A. & CARVALHO, F. (2007) Monoamine oxidase-B mediates ecstasy-induced neurotoxic effects to adolescent rat brain mitochondria. *J. Neurosci.*, 27, 10203-10.

ASKEW, B. M. (1961) Amphetamine toxicity in aggregated mice. *J. Pharm. Pharmacol.*, 13, 701-3.

AZMITIA, E. C. & SEGAL, M. (1978) An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J. Comp. Neurol.*, 179, 641-68.

BADDELEY, A. (1996) The fractionation of working memory. *Proc. Natl. Acad. Sci.*, 93, 13468-13472.

BADDELEY, A. D. & HITCH, G. J. (1974) Working memory. IN BOWER, G. H. (Ed.) The psychology of learning and motivation: Advances in research and theory. New York, Academic Press.

BADON, L. A., HICKS, A., LORD, K., OGDEN, B. A., MELEG-SMITH, S. & VARNER, K. J. (2002) Changes in cardiovascular responsiveness and cardiotoxicity elicited during binge administration of Ecstasy. *J. Pharmacol. Exp. Ther.*, 302, 898-907.

BAI, F., LAU, S. S. & MONKS, T. J. (1999) Glutathione and N-acetylcysteine conjugates of alpha-methyldopamine produce serotonergic neurotoxicity: possible role in methylenedioxyamphetamine-mediated neurotoxicity. *Chem.Res.Toxicol.*, 12, 1150-1157.

BALL, K. T., BUDREAU, D. & REBEC, G. V. (2003) Acute effects of 3,4-methylenedioxymethamphetamine on striatal single-unit activity and behavior in freely moving rats: differential involvement of dopamine D(1) and D(2) receptors. *Brain Res.*, 994, 203-215.

BALL, K. T. & REBEC, G. V. (2005) Role of 5-HT2A and 5-HT2C/B receptors in the acute effects of 3,4-methylenedioxymethamphetamine (MDMA) on striatal single-unit activity and locomotion in freely moving rats. *Psychopharmacology (Berl)*, 181, 676-687.

BANKSON, M. G. & CUNNINGHAM, K. A. (2001) 3,4-Methylenedioxymethamphetamine (MDMA) as a unique model of serotonin receptor function and serotonin-dopamine interactions. *J. Pharmacol. Exp. Ther.*, 297, 846-52.

BANKSON, M. G. & CUNNINGHAM, K. A. (2002) Pharmacological studies of the acute effects of (+)-3,4-methylenedioxymethamphetamine on locomotor activity: role of 5-HT(1B/1D) and 5-HT(2) receptors. *Neuropsychopharmacology*, 26, 40-52.

BARNES, N. M. & SHARP, T. (1999) A review of central 5-HT receptors and their function. *Neuropharmacology*, 38, 1083-1152.

BATTAGLIA, G., SHARKEY, J., KUHAR, M. J. & DE SOUZA, E. B. (1991) Neuroanatomic specificity and time course of alterations in rat brain serotonergic pathways induced by MDMA (3,4-methylenedioxymethamphetamine): assessment using quantitative autoradiography. *Synapse*, 8, 249-260.

BATTAGLIA, G., YEH, S. Y. & DE SOUZA, E. B. (1988) MDMA-induced neurotoxicity: parameters of degeneration and recovery of brain serotonin neurons. *Pharmacol. Biochem. Behav.*, 29, 269-274.

BATTAGLIA, G., YEH, S. Y., O'HEARN, E., MOLLIVER, M. E., KUHAR, M. J. & DE SOUZA, E. B. (1987) 3,4-Methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: quantification of neurodegeneration by measurement of [^{3}H]paroxetine-labeled serotonin uptake sites. *J. Pharmacol. Exp. Ther.*, 242, 911-916.

BAUER, R. H. & FUSTER, J. M. (1978) Effects of d-amphetamine and prefrontal cortical cooling on delayed matching-to-sample behavior. *Pharmacol. Biochem. Behav.*, 8, 243-249.

BAUMANN, M. H., CLARK, R. D., FRANKEN, F. H., RUTTER, J. J. & ROTHMAN, R. B. (2008a) Tolerance to 3,4-methylenedioxymethamphetamine in rats exposed to single high-dose binges. *Neuroscience*, 152, 773-84.

BAUMANN, M. H., CLARK, R. D. & ROTHMAN, R. B. (2008b) Locomotor stimulation produced by 3,4-methylenedioxymethamphetamine (MDMA) is correlated with dialysate levels of serotonin and dopamine in rat brain. *Pharmacology, Biochemistry & Behavior*, 90, 208-217.

BAUMANN, M. H., WANG, X. & ROTHMAN, R. B. (2007) 3,4-Methylenedioxymethamphetamine (MDMA) neurotoxicity in rats: a reappraisal of past and present findings. *Psychopharmacology,* 189, 407-24.

BEAR, M. F., CONNORS, B. W. & PARADISO, M. A. (2006) *Neuroscience Exploring the Brain* New York, Lippincott Williams & Wilkins.

BENAMAR, K., GELLER, E. B. & ADLER, M. W. (2008) A new brain area affected by 3,4-methylenedioxymethamphetamine: A microdialysis-biotelemetry study. *European Journal of Pharmacology*, 596, 84-88.

BENAZZI, F. & MAZZOLI, M. (1991) Psychiatric illness associated with "ecstasy". *Lancet*, 338, 1520.

BERGE, O. G. & OGREN, S. O. (1984) Selective lesions of the bulbospinal serotonergic pathways reduce the analgesia induced by *p*-chloroamphetamine in the hot-plate test. *Neurosci. Lett.*, 44, 25-9.

BERGER, U. V., GU, X. F. & AZMITIA, E. C. (1992) The substituted amphetamines 3,4-methylenedioxymethamphetamine, methamphetamine, *p*-chloroamphetamine and fenfluramine induce 5-hydroxytryptamine release via a common mechanism blocked by fluoxetine and cocaine. *Eur.J.Pharmacol.*, 215, 153-160.

BIGGIO, G., PORCEDDU, M. L. & GEYER, M. (1976) Decrease of homovanillic, dihydroxyphenylacetic acid and cyclic-adenosine-3',5'-monophosphate content in the rat caudate nucleus induced by the acute administration of an aminoacid mixture lacking tyrosine and phenylalanine. *Journal of Neurochemistry*, 26, 1253-1255.

BLESSING, W. W., SEAMAN, B., PEDERSEN, N. P. & OOTSUKA, Y. (2003) Clozapine reverses hyperthermia and sympathetically mediated cutaneous vasoconstriction induced by 3,4-methylenedioxymethamphetamine (ecstasy) in rabbits and rats. *J.Neurosci.*, 23, 6385-6391.

BLIGH, J. (1979) The central neurology of mammalian thermoregulation. *Neuroscience,* 4, 1213-1236.

BOLLA, K. I., MCCANN, U. D. & RICAURTE, G. A. (1998) Memory impairment in abstinent MDMA ("Ecstasy") users. *Neurology*, 51, 1532-1537.

BONGIOVANNI, R., YAMAMOTO, B. K. & JASKIW, G. E. (2001) Improved method for the measurement of large neutral amino acids in biological matrices. *J. Chromatogr. B Biomed. Sci. Appl.*, 754, 369-376.

BRAIDA, D., POZZI, M., CAVALLINI, R. & SALA, M. (2002) 3,4 methylenedioxymethamphetamine (ecstasy) impairs eight-arm radial maze performance and arm entry pattern in rats. *Behav.Neurosci.*, 116, 298-304.

BREIER, J. M., BANKSON, M. G. & YAMAMOTO, B. K. (2006) L-tyrosine contributes to (+)-3,4-methylenedioxymethamphetamine-induced serotonin depletions. *J. Neurosci.*, 26, 290-299.

BREZUN, J. M. & DASZUTA, A. (1999) Depletion in serotonin decreases neurogenesis in dentate gyrus and the subventricular zone of adult rats. *Neuroscience,* 89, 999-1002.

BROENING, H. W., BOWYER, J. F. & SLIKKER, W., JR. (1995) Age-dependent sensitivity of rats to the long-term effects of the serotonergic neurotoxicant (+/-)-3,4-methylenedioxymethamphetamine (MDMA) correlates with the magnitude of the MDMA-induced thermal response. *J. Pharmacol. Exp. Ther.*, 275, 325-333.

BROENING, H. W., MORFORD, L. L., INMAN-WOOD, S. L., FUKUMURA, M. & VORHEES, C. V. (2001) 3,4-methylenedioxymethamphetamine (ecstasy)-induced learning and memory impairments depend on the age of exposure during early development. *J. Neurosci.*, 21, 3228-3235.

BROERSEN, L. M., HEINSBROEK, R. P., DE BRUIN, J. P., UYLINGS, H. B. & OLIVIER, B. (1995) The role of the medial prefrontal cortex of rats in short-term memory functioning: further support for involvement of cholinergic, rather than dopaminergic mechanisms. *Brain Res.*, 674, 221-9.

BROWN, C. & OSTERLOH, J. (1987) Multiple severe complications from recreational ingestion of MDMA ('Ecstasy'). *JAMA*, 258, 780-781.

BROZOWSKI, T. S., BROWN, R. M., ROSVOLD, H. E. & GOLDMAN, P. S. (1979) Cognitive deficits caused by regional depletion of dopamine in prefrontal cortex of Rhesus monkey. *Science*, 205, 929-932.

BRUEL-JUNGERMAN, E., LAROCHE, S. & RAMPON, C. (2005) New neurons in the dentate gyrus are involved in the expression of enhanced long-term memory following environmental enrichment. *Eur.J.Neurosci.*, 21, 513-21.

BUBSER, M. & SCHMIDT, W. J. (1990) 6-Hydroxydopamine lesion of the rat prefrontal cortex increases locomotor activity, impairs acquisition of delayed alternation tasks, but does not affect uninterrupted tasks in the radial maze. *Behav. Brain Res.*, 37, 157-68.

BUCHERT, R., THOMASIUS, R., WILKE, F., PETERSEN, K., NEBELING, B., OBROCKI, J., SCHULZE, O., SCHMIDT, U. & CLAUSEN, M. (2004) A voxel-based PET investigation of the long-term effects of "Ecstasy" consumption on brain serotonin transporters. *Am. J. Psychiatry*, 161, 1181-1189.

CALLAWAY, C. W., JOHNSON, M. P., GOLD, L. H., NICHOLS, D. E. & GEYER, M. A. (1991) Amphetamine derivatives induce locomotor hyperactivity by acting as indirect serotonin agonists. *Psychopharmacology (Berl)*, 104, 293-301.

CALLAWAY, C. W., WING, L. L. & GEYER, M. A. (1990) Serotonin release contributes to the locomotor stimulant effects of 3,4-methylenedioxymethamphetamine in rats. *J. Pharmacol. Exp. Ther.*, 254, 456-464.

CAMARERO, J., SANCHEZ, V., O'SHEA, E., GREEN, A. R. & COLADO, M. I. (2002) Studies, using in vivo microdialysis, on the effect of the dopamine uptake inhibitor GBR 12909 on 3,4-methylenedioxymethamphetamine ('ecstasy')-induced dopamine release and free radical formation in the mouse striatum. *J.Neurochem.*, 81, 961-972.

CAPELA, J. P., CARMO, H., REMIAO, F., BASTOS, M. L., MEISEL, A. & CARVALHO, F. (2009) Molecular and cellular mechanisms of ecstasy-induced neurotoxicity: An overview. *Molecular Neurobiology*.

CAPELA, J. P., MEISEL, A., ABREU, A. R., BRANCO, P. S., FERREIRA, L. M., LOBO, A. M., REMIAO, F., BASTOS, M. L. & CARVALHO, F. (2005) Neurotoxicity of Ecstasy metabolites in rat cortical neurons, and influence of hyperthermia. *J. Pharmacol. Exp. Ther.*, 316, 53-61.

CARLI, M., BALDUCCI, C. & SAMANIN, R. (2001) Stimulation of 5-HT1A receptors in the dorsal raphe ameliorates the impairment of spatial learning caused by intrahippocampal 7-chloro-kynurenic acid in naive and pretrained rats. *Psychopharmacology (Berl)*, 158, 39-47.

CARLI, M., BAVIERA, M., INVERNIZZI, R. W. & BALDUCCI, C. (2006) Dissociable contribution of 5-HT1A and 5-HT2A receptors in themedial prefrontal cortex to different aspects of executive control such as impulsivity and compulsive perseveration in rats. *Neuropsychopharmacology*, 31, 757-67.

CHANCE, M. (1947) Aggregation as a factor influencing the toxicity of sympathomimetic amines in mice. *J. Pharmacol. Exp. Ther.*, 87, 214-219.

CHE, S., JOHNSON, M., HANSON, G. R. & GIBB, J. W. (1995) Body temperature effect on methylenedioxymethamphetamine-induced acute decrease in tryptophan hydroxylase activity. *Eur.J.Pharmacol.*, 293, 447-453.

CHO, K. O., KIM, S. K., RHEE, G. S., KWACK, S. J., CHO, D. H., SUNG, K. W. & KIM, S. Y. (2007) Chronic 3,4-methylenedioxymethamphetamine treatment suppresses cell proliferation in the adult mouse dentate gyrus. *Eur. J. Pharmacol.*, 566, 120-3.

CHUHAN, Y. S. & TAUKULIS, H. K. (2006) Impairment of single-trial memory formation by oral methylphenidat in the rat. *Neurobiol. Learn. Mem.*, 85, 125-131.

COHEN, R. S. (1995) Subjective reports on the effects of the MDMA ('ecstasy') experience in humans. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 19, 1137-1145.

COHEN, R. S. (1996) Adverse symptomatology and suicide associated with the use of methylenedioxymethamphetamine (MDMA; "Ecstasy"). *Biol.Psychiatry*, 39, 819-820.

COLADO, M. I., CAMARERO, J., MECHAN, A. O., SANCHEZ, V., ESTEBAN, B., ELLIOTT, J. M. & GREEN, A. R. (2001) A study of the mechanisms involved in the neurotoxic action of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') on dopamine neurones in mouse brain. *Br.J.Pharmacol.*, 134, 1711-1723.

COLADO, M. I., GRANADOS, R., O'SHEA, E., ESTEBAN, B. & GREEN, A. R. (1999c) The acute effect in rats of 3,4-methylenedioxyethamphetamine (MDEA, "eve") on body temperature and long term degeneration of 5-HT neurones in brain: a comparison with MDMA ("ecstasy"). *Pharmacol. Toxicol.*, 84, 261-266.

COLADO, M. I. & GREEN, A. R. (1994) A study of the mechanism of MDMA ('ecstasy')-induced neurotoxicity of 5-HT neurones using chlormethiazole, dizocilpine and other protective compounds. *Br.J.Pharmacol.*, 111, 131-136.

COLADO, M. I., MURRAY, T. K. & GREEN, A. R. (1993) 5-HT loss in rat brain following 3,4-methylenedioxymethamphetamine (MDMA), p-chloroamphetamine and fenfluramine administration and effects of chlormethiazole and dizocilpine. *Br.J.Pharmacol.*, 108, 583-589.

COLADO, M. I., O'SHEA, E., ESTEBAN, B., GRANADOS, R. & GREEN, A. R. (1999a) In vivo evidence against clomethiazole being neuroprotective against MDMA ('ecstasy')-induced degeneration of rat brain 5-HT nerve terminals by a free radical scavenging mechanism. *Neuropharmacology*, 38, 307-314.

COLADO, M. I., O'SHEA, E., GRANADOS, R., ESTEBAN, B., MARTIN, A. B. & GREEN, A. R. (1999b) Studies on the role of dopamine in the degeneration of 5-HT nerve endings in the brain of Dark Agouti rats following 3,4-methylenedioxymethamphetamine (MDMA or 'ecstasy') administration. *Br. J. Pharmacol.*, 126, 911-924.

COLADO, M. I., O'SHEA, E., GRANADOS, R., MURRAY, T. K. & GREEN, A. R. (1997) In vivo evidence for free radical involvement in the degeneration of rat brain 5-HT following administration of MDMA ('ecstasy') and p-chloroamphetamine but not the degeneration following fenfluramine. *Br. J. Pharmacol.*, 121, 889-900.

COLADO, M. I., WILLIAMS, J. L. & GREEN, A. R. (1995) The hyperthermic and neurotoxic effects of 'Ecstasy' (MDMA) and 3,4 methylenedioxyamphetamine (MDA)

in the Dark Agouti (DA) rat, a model of the CYP2D6 poor metabolizer phenotype. 115, 1281-1289.

COLE, J. C., BAILEY, M., SUMNALL, H. R., WAGSTAFF, G. F. & KING, L. A. (2002) The content of ecstasy tablets: implications for the study of their long-term effects. *Addiction*, 97, 1531-1536.

COMMINS, D. L., VOSMER, G., VIRUS, R. M., WOOLVERTON, W. L., SCHUSTER, C. R. & SEIDEN, L. S. (1987) Biochemical and histological evidence that methylenedioxymethylamphetamine (MDMA) is toxic to neurons in the rat brain. *J. Pharmacol. Exp. Ther.*, 241, 338-345.

CONNOLLY, E. & O'CALLAGHAN, G. (1999) MDMA toxicity presenting with severe hyperpyrexia: a case report. *Crit Care Resusc.*, 1, 368-370.

CONSOLO, S., ARNABOLDI, S., GIORGI, S., RUSSI, G. & LADINSKY, H. (1994) 5-HT4 receptor stimulation facilitates acetylcholine release in rat frontal cortex. *Neuroreport*, 5, 1230-2.

COWAN, R. L. (2007) Neuroimaging research in human MDMA users: a review. *Psychopharmacology*, 189, 539-56.

COWAN, R. L., WILSON, C. J., EMSON, P. C. & HEIZMANN, C. W. (1990) Parvalbumin-containing GABAergic interneurons in the rat neostriatum. *J. Comp. Neurol.*, 302, 197-205.

CRAIG, A. L. & KUPFERBERG, H. J. (1972) Hyperthermia in d-amphetamine toxicity in aggregated mice of different strains. *J. Pharmacol. Exp. Ther.*, 180, 616-24.

CREIGHTON, F. J., BLACK, D. L. & HYDE, C. E. (1991) 'Ecstasy' psychosis and flashbacks. *Br.J.Psychiatry*, 159, 713-715.

CURRAN, H. V. (2000) Is MDMA ('Ecstasy') neurotoxic in humans? An overview of evidence and of methodological problems in research. . *Neuropsychobiology*, 42, 34-41.

CURRAN, H. V. & TRAVILL, R. A. (1997) Mood and cognitive effects of +/-3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy'): week-end 'high' followed by mid-week low. *Addiction*, 92, 821-831.

DAFTERS, R. I. (1994) Effect of ambient temperature on hyperthermia and hyperkinesis induced by 3,4-methylenedioxymethamphetamine (MDMA or "ecstasy") in rats. *Psychopharmacology (Berl)*, 114, 505-508.

DAFTERS, R. I. (1995) Hyperthermia following MDMA administration in rats: effects of ambient temperature, water consumption, and chronic dosing. *Physiol. Behav.*, 58, 877-882.

DAFTERS, R. I. & LYNCH, E. (1998) Persistent loss of thermoregulation in the rat induced by 3,4-methylenedioxymethamphetamine (MDMA or "Ecstasy") but not by fenfluramine. *Psychopharmacology (Berl)*, 138, 207-212.

DAVIS, W. M. & BORNE, R. F. (1984) Pharmacologic investigation of compounds related to 3,4-methylenedioxyamphetamine (MDA). *Subst.Alcohol Actions Misuse.*, 5, 105-110.

DAVISON, D. & PARROTT, A. C. (1997) Ecstasy in recreation users: self-reported psychological and physiological effects. *Hum. Psychopharmacol.*, 12, 91-97.

DE LA TORRE, R. & FARRE, M. (2004) Neurotoxicity of MDMA (ecstasy): the limitations of scaling from animals to humans. *Trends Pharmacol. Sci.*, 25, 505-8.

DE LA TORRE, R., FARRE, M., ORTUNO, J., MAS, M., BRENNEISEN, R., ROSET, P. N., SEGURA, J. & CAMI, J. (2000) Non-linear pharmacokinetics of MDMA ('ecstasy') in humans. *Br. J. Clin. Pharmacol.*, 49, 104-109.

DE WIN, M. M., DE JEU, R. A., DE, B. K., HABRAKEN, J. B., RENEMAN, L., BOOIJ, J. & DEN HEETEN, G. J. (2004) Validity of in vivo [123I]beta-CIT SPECT in detecting MDMA-induced neurotoxicity in rats. *Eur.Neuropsychopharmacol.*, 14, 185-189.

DELAFORGE, M., JAOUEN, M. & BOUILLE, G. (1999) Inhibitory metabolite complex formation of methylendioxymethamphetamine with rat and human

cytochrome P450. Particular involvement of CYP2D. *Environ. Toxicol. Pharmaco.*, 7, 153-158.

DIX, S. L. & AGGLETON, J. P. (1999) Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. *Behav. Brain Res.*, 99, 191-200.

DJAVADIAN, R. L. (2004) Serotonin and neurogenesis in the hippocampal dentate gyrus of adult mammals. *Acta Neurobiol Exp,* 64, 189-200.

DOMINGUEZ-ESCRIBA, L., HERNANDEZ-RABAZA, V., SORIANO-NAVARRO, M., BARCIA, J. A., ROMERO, F. J., GARCIA-VERDUGO, J. M. & CANALES, J. J. (2006) Chronic cocaine exposure impairs progenitor proliferation but spares precusor maturation in adult rat dentate gyrus. *Eur.J.Neurosci.*, 24, 586-594.

DURKIN, S., PRENDERGAST, A. & HARKIN, A. (2008) Reduced efficacy of fluoxetine following MDMA ("Ecstasy")-induced serotonin loss in rats. Progress in Neuro-Psychopharmacology Psychiatry, 32, 1894-1901.

EASTON, N., FRY, J., O'SHEA, E., WATKINS, A., KINGSTON, S. & MARSDEN, C. A. (2003) Synthesis, in vitro formation, and behavioural effects of glutathione regioisomers of alpha-methyldopamine with relevance to MDA and MDMA (ecstasy). *Brain Res.*, 987, 144-154.

EASTON, N. & MARSDEN, C. A. (2006) Ecstasy: are animal data consistent between species and can they translate to humans? *J.Psychopharmacol.*, 20, 194-210.

EMCDDA (2007) *Annual report 2007: the state of drugs problem in Europe*, Lisbon, European Monitoring Centre for Drugs and Drug Addiction.

ENNACEUR, A. & DELACOUR, J. (1988) A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behavioural Brain Research*, 31, 47-59.

ENNACEUR, A. & MELIANI, K. (1992) Effects of physostigmine and scopolamine on rats' performances in object-recognition and radial-maze tests. *Psychopharmacology*, 109, 321-330.

ENNACEUR, A., MICHALIKOVA, S., BRADFORD, A. & AHMED, S. (2005) Detailed analysis of the behavior of Lister and Wistar rats in anxiety, object recognition and object location tasks. Behav Brain Res, 159, 247-266.

ESCOBEDO, I., O'SHEA, E., ORIO, L., SANCHEZ, V., SEGURA, M., DE LA, T. R., FARRE, M., GREEN, A. R. & COLADO, M. I. (2005) A comparative study on the acute and long-term effects of MDMA and 3,4-dihydroxymethamphetamine (HHMA) on brain monoamine levels after i.p. or striatal administration in mice. *British Journal of Pharmacology*, 144, 231-41.

ESTEBAN, B., O'SHEA, E., CAMARERO, J., SANCHEZ, V., GREEN, A. R. & COLADO, M. I. (2001) 3,4-Methylenedioxymethamphetamine induces monoamine release, but not toxicity, when administered centrally at a concentration occurring following a peripherally injected neurotoxic dose. *Psychopharmacology*, 154, 251-60.

FALK, E. M., COOK, V. J., NICHOLS, D. E. & SPRAGUE, J. E. (2002) An antisense oligonucleotide targeted at MAO-B attenuates rat striatal serotonergic neurotoxicity induced by MDMA. *Pharmacol.Biochem.Behav.*, 72, 617-622.

FANTEGROSSI, W. E., GODLEWSKI, T., KARABENICK, R. L., STEPHENS, J. M., ULLRICH, T., RICE, K. C. & WOODS, J. H. (2003) Pharmacological characterization of the effects of 3,4-methylenedioxymethamphetamine ("ecstasy") and its enantiomers on lethality, core temperature, and locomotor activity in singly housed and crowded mice. *Psychopharmacology (Berl)*, 166, 202-211.

FARAJ, B. A., OLKOWSKI, Z. L. & JACKSON, R. T. (1994) Active [3H]-dopamine uptake by human lymphocytes: Correlates with serotonin transporter activity. *Pharmacol.*, 48, 320-327.

FARFEL, G. M. & SEIDEN, L. S. (1995) Role of hypothermia in the mechanism of protection against serotonergic toxicity. I. Experiments using 3,4-methylenedioxymethamphetamine, dizocilpine, CGS 19755 and NBQX. *J. Pharmacol. Exp. Ther.*, 272, 860-867.

FARRE, M., DE LA, T. R., MATHUNA, B. O., ROSET, P. N., PEIRO, A. M., TORRENS, M., ORTUNO, J., PUJADAS, M. & CAMI, J. (2004) Repeated doses

administration of MDMA in humans: pharmacological effects and pharmacokinetics. *Psychopharmacology*, 173, 364-75.

FERNSTROM, M. H. & FERNSTROM, J. D. (1995) Acute tyrosine depletion reduces tyrosine hydroxylation rate in rat central nervous system. *Life Sciences*, 57, 97-102.

FERNSTROM, M. H. & WURTMANM, R. J. (1972) Brain serotonin content: physiological regulation by plasma neutral amino acids. *Science*, 178, 414-416.

FISCHER, C., HATZIDIMITRIOU, G., WLOS, J., KATZ, J. & RICAURTE, G. (1995) Reorganization of ascending 5-HT axon projections in animals previously exposed to the recreational drug (+/-)3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). *J.Neurosci.*, 15, 5476-5485.

FISCHER, H. S., ZERNIG, G., SCHATZ, D. S., HUMPEL, C. & SARIA, A. (2000) MDMA ('ecstasy') enhances basal acetylcholine release in brain slices of the rat striatum. *Eur.J.Neurosci.*, 12, 1385-1390.

FISCHMAN, M. W. & JOHANSON, C. E. (1996) Cocaine. IN SCHUSTER, C. R. & KUHAR, M. J. (Eds.) *Pharmacological Aspects of Drug Dependence: Towards an Integrated Neurobehavioral Approach*. New York, Springer.

FITZGERALD, J. L. & REID, J. J. (1990) Effects of methylenedioxymethamphetamine on the release of monoamines from rat brain slices. *Eur.J.Pharmacol.*, 191, 217-220.

FITZGERALD, J. L. & REID, J. J. (1993) Interactions of methylenedioxymethamphetamine with monoamine transmitter release mechanisms in rat brain slices. *Naunyn Schmiedebergs Arch.Pharmacol.*, 347, 313-323.

FITZGERALD, J. L. & REID, J. J. (1994) Sympathomimetic actions of methylenedioxymethamphetamine in rat and rabbit isolated cardiovascular tissues. *J.Pharm.Pharmacol.*, 46, 826-832.

FLETCHER, P. J., KORTH, K. M., ROBINSON, S. R. & BAKER, G. B. (2002) Multiple 5-HT receptors are involved in the effects of acute MDMA treatment: studies

on locomotor activity and responding for conditioned reinforcement. *Psychopharmacology (Berl)*, 162, 282-291.

FLETCHER, P. J., SINYARD, J. & HIGGINS, G. A. (2006) The effects of the 5-HT(2C) receptor antagonist SB242084 on locomotor activity induced by selective, or mixed, indirect serotonergic and dopaminergic agonists. *Psychopharmacology*, 187, 515-25.

FLORESCO, S. B. & MAGYAR, O. (2006) Mesocortical dopamine modulation of executive functions: beyond working memory. *Psychopharmacology*, 188, 567-585.

FONE, K. C., BECKETT, S. R., TOPHAM, I. A., SWETTENHAM, J., BALL, M. & MADDOCKS, L. (2002) Long-term changes in social interaction and reward following repeated MDMA administration to adolescent rats without accompanying serotonergic neurotoxicity. *Psychopharmacology (Berl)*, 159, 437-444.

FONTANA, D. J., DANIELS, S. E., WONG, E. H., CLARK, R. D. & EGLEN, R. M. (1997) The effects of novel, selective 5-hydroxytryptamine (5-HT)4 receptor ligands in rat spatial navigation. *Neuropharmacology*, 36, 689-96.

FORSTER, E. A., CLIFFE, I. A., BILL, D. J., DOVER, G. M., JONES, D., REILLY, Y. & FLETCHER, A. (1995) A pharmacological profile of the selective silent 5-HT1A receptor antagonist, WAY-100635. *Eur.J.Pharmacol.*, 281, 81-88.

FOX, H. C., MCLEAN, A., TURNER, J. J., PARROTT, A. C., ROGERS, R. & SAHAKIAN, B. J. (2002) Neuropsychological evidence of a relatively selective profile of temporal dysfunction in drug-free MDMA ("ecstasy") polydrug users. *Psychopharmacology (Berl)*, 162, 203-214.

FOX, H. C., PARROTT, A. C. & TURNER, J. J. (2001) Ecstasy use: cognitive deficits related to dosage rather than self-reported problematic use of the drug. *J. Psychopharmacol.*, 15, 273-281.

FREDERICK, D. L., ALI, S. F., GILLAM, M. P., GOSSETT, J., SLIKKER, W. & PAULE, M. G. (1998) Acute effects of dexfenfluramine (d-FEN) and methylenedioxymethamphetamine (MDMA) before and after short-course, high-dose treatment. *Ann.N.Y.Acad.Sci.*, 844, 183-190.

FREDERICK, D. L. & PAULE, M. G. (1997) Effects of MDMA on complex brain function in laboratory animals. *Neurosci.Biobehav.Rev.*, 21, 67-78.

FREUDENMANN, R. W., OXLER, F. & BERNSCHNEIDER-REIF, S. (2006) The origin of MDMA (ecstasy) revisited: the true story reconstructed from the original documents. *Addiction*, 101, 1241-5.

GALEOTTI, N., GHELARDINI, C. & BARTOLINI, A. (1998) Role of 5-HT4 receptors in the mouse passive avoidance test. *J. Pharmacol. Exp. Ther.*, 286, 115-21.

GERRA, G., ZAIMOVIC, A., FERRI, M., ZAMBELLI, U., TIMPANO, M., NERI, E., MARZOCCHI, G. F., DELSIGNORE, R. & BRAMBILLA, F. (2000) Long-lasting effects of (+/-)3,4-methylenedioxymethamphetamine (ecstasy) on serotonin system function in humans. *Biol. Psychiatry*, 47, 127-136.

GERRA, G., ZAIMOVIC, A., GIUCASTRO, G., MAESTRI, D., MONICA, C., SARTORI, R., CACCAVARI, R. & DELSIGNORE, R. (1998) Serotonergic function after (+/-)3,4-methylene-dioxymethamphetamine ('Ecstasy') in humans. *Int. Clin. Psychopharmacol.*, 13, 1-9.

GIBB, J. W., JOHNSON, M. & HANSON, G. R. (1990) Neurochemical basis of neurotoxicity. *Neurotoxicology*, 11, 317-321.

GILLMAN, P. K. (1999) The serotonin syndrome and its treatment. *J. Psychopharmacol.*, 13, 100-9.

GOBERT, A., RIVET, J. M., LEJEUNE, F., NEW-MAN-TANCREDI, A., ADHUMEAU-AUCLAIR, A., NICOLAS, J. P., CISTARELLI, L., MELON, C. & MILLAN, M. J. (2000) Serotonin2C receptors tonically suppress the activity of mesocortical dopaminergic and adrenergic, but not serotonergic, pathways: a combined dialysis and electrophysiological analysis in the rat. *Synapse*, 36, 205-221.

GOLD, L. H., HUBNER, C. B. & KOOB, G. F. (1989) A role for the mesolimbic dopamine system in the psychostimulant actions of MDMA. *Psychopharmacology (Berl)*, 99, 40-47.

GOLD, L. H., KOOB, G. F. & GEYER, M. A. (1988) Stimulant and hallucinogenic behavioral profiles of 3,4-methylenedioxymethamphetamine and N-ethyl-3,4-methylenedioxyamphetamine in rats. *J. Pharmacol. Exp. Ther.*, 247, 547-555.

GONI-ALLO, B., PUERTA, E., MATHUNA, B. O., HERVIAS, I., LASHERAS, B., DE LA, T. R. & AGUIRRE, N. (2008) On the role of tyrosine and peripheral metabolism in 3,4-methylenedioxymethamphetamine-induced serotonin neurotoxicity in rats. *Neuropharmacology*, 54, 885-900.

GORDON, C. J., WATKINSON, W. P., O'CALLAGHAN, J. P. & MILLER, D. B. (1991) Effects of 3,4-methylenedioxymethamphetamine on autonomic thermoregulatory responses of the rat. *Pharmacol. Biochem. Behav.*, 38, 339-344.

GOUGH, B., ALI, S. F., SLIKKER, W., JR. & HOLSON, R. R. (1991) Acute effects of 3,4-methylenedioxymethamphetamine (MDMA) on monoamines in rat caudate. *Pharmacol. Biochem. Behav.*, 39, 619-623.

GOUZOULIS-MAYFRANK, E., DAUMANN, J., TUCHTENHAGEN, F., PELZ, S., BECKER, S., KUNERT, H. J., FIMM, B. & SASS, H. (2000) Impaired cognitive performance in drug free users of recreational ecstasy (MDMA). *Journal of Neurology, Neurosurgery & Psychiatry*, 68, 719-25.

GRAHAME-SMITH, D. G. & PARFITT, A. (1970) Tryptophan transport across the synaptosomal membrane. *J. Neurochem.*, 17, 1339-1353.

GREEN, A. R. (2006) Neuropharmacology of 5-hydroxytryptamine. *Br. J. Pharmacol.*, 147 Suppl 1, S145-52.

GREEN, A. R., GABRIELSSON, J., MARSDEN, C. A. & FONE, K. C. (2009) MDMA: On the translation from rodent to human dosing. *Psychopharmacology (Berl)*, 204, 375-8.

GREEN, A. R., MECHAN, A. O., ELLIOTT, J. M., O'SHEA, E. & COLADO, M. I. (2003) The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). Pharmacol.Rev., 55, 463-508.

GREEN, A. R., O'SHEA, E. & COLADO, M. I. (2004) A review of the mechanisms involved in the acute MDMA (ecstasy)-induced hyperthermic response. *European Journal of Pharmacology,* 500, 3-13.

GREEN, A. R., O'SHEA, E., SAADAT, K. S., ELLIOTT, J. M. & COLADO, M. I. (2005) Studies on the effect of MDMA ('ecstasy') on the body temperature of rats housed at different ambient room temperatures. *Br. J. Pharmacol.,* 146, 306-12.

GREEN, A. R., SANCHEZ, V., O'SHEA, E., SAADAT, K. S., ELLIOTT, J. M. & COLADO, M. I. (2004) Effect of ambient temperature and a prior neurotoxic dose of 3,4-methylenedioxymethamphetamine (MDMA) on the hyperthermic response of rats to a single or repeated ('binge' ingestion) low dose of MDMA. *Psychopharmacology,* 173, 264-9.

GREENBLATT, E. & OSTERBERG, A. (1961) Correlations of activating and lethal effects of excitatory drugs in grouped and isolated mice. *J. Pharmacol. Exp. Ther.,* 131, 115-119.

GUDELSKY, G. A. (1996) Effect of ascorbate and cysteine on the 3,4-methylenedioxymethamphetamine-induced depletion of brain serotonin. *J. Neural Transm.*, 103, 1397-1404.

GUDELSKY, G. A. & NASH, J. F. (1996) Carrier-mediated release of serotonin by 3,4-methylenedioxymethamphetamine: implications for serotonin-dopamine interactions. *J. Neurochem.*, 66, 243-249.

HAMMERSLEY, R., DITTON, J., SMITH, I. & SHORT, E. (1999) Patterns of Ecstasy use by drug users. *Br. J. Criminol,* 39, 625-647.

HARDER, J. A. & RIDLEY, R. M. (2000) The 5-HT1A antagonist, WAY 100 635, alleviates cognitive impairments induced by dizocilpine (MK-801) in monkeys. *Neuropharmacology,* 39, 547-552.

HARPER, D. N., HUNT, M. & SCHENK, S. (2006) Attenuation of the disruptive effects of (+/-)3,4-methylene dioxymethamphetamine (MDMA) on delayed matching-to-sample performance in the rat. *Behav. Neurosci.,* 120, 201-205.

HARPER, D. N., WISNEWSKI, R., HUNT, M. & SCHENK, S. (2005) (+/-)3,4-methylenedioxymethamphetamine, d-amphetamine, and cocaine impair delayed matching-to-sample performance by an increase in susceptibility to proactive interference. *Behav. Neurosci.*, 119, 455-463.

HATZIDIMITRIOU, G., MCCANN, U. D. & RICAURTE, G. A. (1999) Altered serotonin innervation patterns in the forebrain of monkeys treated with (+/-)3,4-methylenedioxymethamphetamine seven years previously: factors influencing abnormal recovery. *J. Neurosci.*, 19, 5096-5107.

HEKMATPANAH, C. R., MCKENNA, D. J. & PEROUTKA, S. J. (1989) Reserpine does not prevent 3,4-methylenedioxymethamphetamine-induced neurotoxicity in the rat. *Neurosci. Lett.*, 104, 178-182.

HELMLIN, H. J., BRACHER, K., BOURQUIN, D., VONLANTHEN, D. & BRENNEISEN, R. (1996) Analysis of 3,4-methylenedioxymethamphetamine (MDMA) and its metabolites in plasma and urine by HPLC-DAD and GC-MS. *J.Anal.Toxicol.*, 20, 432-440.

HENRY, J. A. (1996) Ecstasy and serotonin depletion. Lancet, 347, 833.

HERIN, D. V., LIU, S., ULLRICH, T., RICE, K. C. & CUNNINGHAM, K. A. (2005) Role of the serotonin 5-HT2A receptor in the hyperlocomotive and hyperthermic effects of (+)-3,4-methylenedioxymethamphetamine. *Psychopharmacology (Berl)*, 178, 505-513.

HERNANDEZ-RABAZA, V., DOMINGUEZ-ESCRIBA, L., BARCIA, J. A., ROSEL, J. F., ROMERO, F. J., GARCIA-VERDUGO, J. M. & CANALES, J. J. (2006) Binge administration of 3,4-methylenedioxymethamphetamine ("ecstasy") impairs the survival of neural precursors in adult rat dentate gyrus. *Neuropharmacology*, 51, 967-73.

HEWITT, K. E. & GREEN, A. R. (1994) Chlormethiazole, dizocilpine and haloperidol prevent the degeneration of serotonergic nerve terminals induced by administration of MDMA ('Ecstasy') to rats. *Neuropharmacology*, 33, 1589-1595.

HIRST, W. D., ANDREE, T. H., ASCHMIES, S., CHILDERS, W. E., COMERY, T. A., DAWSON, L. A., DAY, M., FEINGOLD, I. B., GRAUER, S. M., HARRISON, B. L., HUGHES, Z. A., KAO, J., KELLY, M. G., VAN DER LEE, H., ROSENZWEIG-LIPSON, S., SAAB, A. L., SMITH, D. L., SULLIVAN, K., RIZZO, S. J., TIO, C., ZHANG, M. Y. & SCHECHTER, L. E. (2008) Correlating efficacy in rodent cognition models with in vivo 5-hydroxytryptamine1a receptor occupancy by a novel antagonist, (R)-N-(2-methyl-(4-indolyl-1-piperazinyl)ethyl)-N-(2-pyridinyl)-cyclohexane carboxamide (WAY-101405). *J. Pharmacol. Exp. Ther.*, 325, 134-45.

HIRST, W. D., STEAN, T. O., ROGERS, D. C., SUNTER, D., PUGH, P., MOSS, S. F., BROMIDGE, S. M., RILEY, G., SMITH, D. R., BARTLETT, S., HEIDBREDER, C. A., ATKINS, A. R., LACROIX, L. P., DAWSON, L. A., FOLEY, A. G., REGAN, C. M. & UPTON, N. (2006) SB-399885 is a potent, selective 5-HT6 receptor antagonist with cognitive enhancing properties in aged rat water maze and novel object recognition models. *Eur. J. Pharmacol.*, 553, 109-19.

HO, Y. J., PAWLAK, C. R., GUO, L. & SCHWARTING, R. K. (2004) Acute and long-term consequences of single MDMA administration in relation to individual anxiety levels in the rat. *Behav. Brain Res.*, 149, 135-144.

HOLMES, A., LACHOWICZ, J. E. & SIBLEY, D. R. (2004) Phenotypic analysis of dopamine receptor knockout mice: recent insights into the functional specificity of dopamine receptor subtypes. *Neuropharmacology*, 47, 1117-1134.

HOTTE, M., NAUDON, L. & JAY, T. M. (2005) Modulation of recognition and temporal order memory retrieval by dopamine D1 receptor in rats. Neurobiol. *Learn. Mem.*, 84, 85-92.

HOYER, D., CLARKE, D. E., FOZARD, J. R., HARTIG, P. R., MARTIN, G. R., MYLECHARANE, E. J., SAXENA, P. R. & HUMPHREY, P. P. (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol. Rev.*, 46, 157-203.

HOYER, D., HANNON, J. P. & MARTIN, G. R. (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol. Biochem. Behav.*, 71, 533-554.

HROMETZ, S. L., BROWN, A. W., NICHOLS, D. E. & SPRAGUE, J. E. (2004) 3,4-methylenedioxymethamphetamine (MDMA, ecstasy)-mediated production of hydrogen peroxide in an in vitro model: the role of dopamine, the serotonin-reuptake transporter, and monoamine oxidase-B. *Neurosci. Lett.*, 367, 56-59.

HUETHER, G., ZHOU, D. & RUTHER, E. (1997) Causes and consequences of the loss of serotonergic presynapses elicited by the consumption of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") and its congeners. *J. Neural Transm.*, 104, 771-794.

INSEL, T. R., BATTAGLIA, G., JOHANNESSEN, J. N., MARRA, S. & DE SOUZA, E. B. (1989) 3,4-Methylenedioxymethamphetamine ("ecstasy") selectively destroys brain serotonin terminals in rhesus monkeys. *J. Pharmacol. Exp. Ther.*, 249, 713-720.

IRVINE, R. J., KEANE, M., FELGATE, P., MCCANN, U. D., CALLAGHAN, P. D. & WHITE, J. M. (2006) Plasma drug concentrations and physiological measures in 'dance party' participants. *Neuropsychopharmacology*, 31, 424-430.

IZQUIERDO, I., MEDINA, J. H., IZQUIERDO, L. A., BARROS, D. M., DE SOUZA, M. M. & MELLO E SOUZA, T. (1998) Short- and long-term memory are differentially regulated by monoaminergic systems in the rat brain. *Neurobiol. Learn. Mem.*, 69, 219-24.

IZQUIERDO, I., MEDINA, J. H., VIANNA, M. R., IZQUIERDO, L. A. & BARROS, D. M. (1999) Separate mechanisms for short- and long-term memory. *Behav. Brain Res.*, 103, 1-11.

JACOBS, W. A. (1987) o-Phthalaldehyde-sulfite derivatization of primary amines for liquid chromatography-electrochemistry. *J. Chromatogr.*, 392, 435-41.

JACOBS, B. L., MARTIN-CORA, F. J. & FORNAL, C. A. (2002) Activity of Medullary serotonergic neurons in freely moving animals. *Brain Res Rev*, 40, 45-52.

JAEHNE, E. J., SALEM, A. & IRVINE, R. J. (2005) Effects of 3,4-methylenedioxymethamphetamine and related amphetamines on autonomic and be havioral thermoregulation. *Pharmacol. Biochem. Behav.*, 81, 485-496.

JASKIW, G. E. & BONGIOVANNI, R. (2004) Brain tyrosine depletion attenuates haloperidol-induced striatal dopamine release in vivo and augments haloperidol-induced catalepsy in the rat. *Psychopharmacology*, 172, 100-107.

JOHNSON, M., MITROS, K., STONE, D. M., ZOBRIST, R., HANSON, G. R. & GIBB, J. W. (1992) Effect of flunarizine and nimodipine on the decrease in tryptophan hydroxylase activity induced by methamphetamine and 3,4-methylenedioxymethamphetamine. *J. Pharmacol. Exp. Ther.*, 261, 586-591.

JONES, D. C., DUVAUCHELLE, C., IKEGAMI, A., OLSEN, C. M., LAU, S. S., DE LA, T. R. & MONKS, T. J. (2005) Serotonergic neurotoxic metabolites of ecstasy identified in rat brain. *J. Pharmacol. Exp. Ther.*, 313, 422-431.

KANKAANPAA, A., MERIRINNE, E., LILLSUNDE, P. & SEPPALA, T. (1998) The acute effects of amphetamine derivatives on extracellular serotonin and dopamine levels in rat nucleus accumbens. *Pharmacol. Biochem. Behav.*, 59, 1003-1009.

KEBABIAN, J. W. & CALNE, D. B. (1979) Multiple receptors for dopamine. *Nature*, 277, 93-96.

KEHNE, J. H., KETTELER, H. J., MCCLOSKEY, T. C., SULLIVAN, C. K., DUDLEY, M. W. & SCHMIDT, C. J. (1996) Effects of the selective 5-HT2A receptor antagonist MDL 100,907 on MDMA-induced locomotor stimulation in rats. *Neuropsychopharmacology*, 15, 116-124.

KESNER, R. P., BIERLEY, R. A. & PEBBLES, P. (1981) Short-term memory: the role of d-amphetamine. *Pharmacol Biochem Behav*, 15, 673-676.

KINDLUNDH-HOGBERG, A. M., SCHIOTH, H. B. & SVENNINGSSON, P. (2007) Repeated intermittent MDMA binges reduce DAT density in mice and SERT density in rats in reward regions of the adolescent brain. *Neurotoxicology*, 28, 1158-69.

KING, M. V., MARSDEN, C. A. & FONE, K. C. F. (2008) A role for the 5-HT1A, 5-HT4 and 5-HT6 receptors in learning and memory. *Trends Pharmacol Sci.*, 29, 482-92.

KING, M. V., SLEIGHT, A. J., WOOLLEY, M. L., TOPHAM, I. A., MARSDEN, C. A. & FONE, K. C. (2004) 5-HT6 receptor antagonists reverse delay-dependent deficits in novel object discrimination by enhancing consolidation--an effect sensitive to NMDA receptor antagonism. *Neuropharmacology*, 47, 195-204.

KITA, H., KOSAKA, T. & HEIZMANN, C. W. (1990) Parvalbumin-immunoreactive neurons in rat neostriatum: a light and electron microscopic study. *Brain Res.*, 536, 1-15.

KOCH, S. & GALLOWAY, M. P. (1997) MDMA induced dopamine release in vivo: role of endogenous serotonin. *J. Neural Transm.*, 104, 135-146.

KRAMER, K. & KINTER, L. B. (2003) Evaluation and applications of radiotelemetry in small laboratory animals. *Physiol Genomics,* 13, 197-205.

LAMIRAULT, L. & SIMON, H. (2001) Enhancement of place and object recognition memory in young adult and old rats by RS 67333, a partial agonist of 5-HT4 receptors. *Neuropharmacology*, 41, 844-53.

LAROCHE, S., DAVIS, S. & JAY, T. M. (2000) Plasticity at hippocampal to prefrontal cortex synapses: dual roles in working memory and consolidation. Hippocampus, 10, 438-446.

LAVELLE, A., HONNER, V. & DOCHERTY, J. R. (1999) Investigation of the prejunctional alpha2-adrenoceptor mediated actions of MDMA in rat atrium and vas deferens. *Br. J. Pharmacol.*, 128, 975-980.

LEONARDI, E. T. & AZMITIA, E. C. (1994) MDMA (ecstasy) inhibition of MAO type A and type B: comparisons with fenfluramine and fluoxetine (Prozac). *Neuropsychopharmacology*, 10, 231-238.

LIEBEN, C. K., VAN OORSOUW, K., DEUTZ, N. E. & BLOKLAND, A. (2004) Acute tryptophan depletion induced by a gelatin-based mixture impairs object memory but not affective behavior and spatial learning in the rat. *Behav Brain Res*, 151, 53-64.

LIECHTI, M. E., SAUR, M. R., GAMMA, A., HELL, D. & VOLLENWEIDER, F. X. (2000) Psychological and physiological effects of MDMA ("Ecstasy") after

pretreatment with the 5-HT(2) antagonist ketanserin in healthy humans. *Neuropsychopharmacology*, 23, 396-404.

LIECHTI, M. E. & VOLLENWEIDER, F. X. (2000) The serotonin uptake inhibitor citalopram reduces acute cardiovascular and vegetative effects of 3,4-methylenedioxymethamphetamine ('Ecstasy') in healthy volunteers. *J. Psychopharmacol.*, 14, 269-274.

LIN, H. Q., BURDEN, P. M., CHRISTIE, M. J. & JOHNSTON, G. A. (1999) The anxiogenic-like and anxiolytic-like effects of MDMA on mice in the elevated plus-maze: a comparison with amphetamine. *Pharmacol. Biochem. Behav.*, 62, 403-408.

LOGAN, B. J., LAVERTY, R., SANDERSON, W. D. & YEE, Y. B. (1988) Differences between rats and mice in MDMA (methylenedioxymethylamphetamine) neurotoxicity. *Eur. J. Pharmacol.*, 152, 227-234.

LUCAS, G. & SPAMPINATO, U. (2000) Role of striatal serotonin2A and serotonin2C receptor subtypes in the control of in vivo dopamine outflow in the rat striatum. *J. Neurochem.*, 74, 693-701.

LUDWIG, V., MIHOV, Y. & SCHWARTING, R. K. (2008) Behavioral and neurochemical consequences of multiple MDMA administrations in the rat: role of individual differences in anxiety-related behavior. *Behav. Brain Res.*, 189, 52-64.

LUTTGEN, M., ELVANDER, E., MADJID, N. & OGREN, S. O. (2005) Analysis of the role of 5-HT1A receptors in spatial and aversive learning in the rat. *Neuropharmacology*, 48, 830-852.

MADJID, N., TOTTIE, E. E., LÜTTGEN, M., MEISTER, B., SANDIN, J., KUZMIN, A., STIEDL, O. & OGREN, S. O. (2006) 5-Hydroxytryptamine 1A receptor blockade facilitates aversive learning in mice: interactions with cholinergic and glutamatergic mechanisms. *J. Pharmacol. Exp. Ther.*, 316, 581-91.

MALBERG, J. E., EISCH, A. J., NESTLER, E. J. & DUMAN, R. S. (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J Neurosci*, 20, 9104-10.

MALBERG, J. E., SABOL, K. E. & SEIDEN, L. S. (1996) Co-administration of MDMA with drugs that protect against MDMA neurotoxicity produces different effects on body temperature in the rat. *J. Pharmacol. Exp. Ther.*, 278, 258-267.

MALBERG, J. E. & SEIDEN, L. S. (1998) Small changes in ambient temperature cause large changes in 3,4-methylenedioxymethamphetamine (MDMA)-induced serotonin neurotoxicity and core body temperature in the rat. *J. Neurosci.*, 18, 5086-5094.

MALLICK, A. & BODENHAM, A. R. (1997) MDMA induced hyperthermia: a survivor with an initial body temperature of 42.9 degrees C. *J. Accid. Emerg. Med.*, 14, 336-338.

MARCHETTI, E., CHAILLAN, F. A., DUMUIS, A., BOCKAERT, J., SOUMIREU-MOURAT, B. & ROMAN, F. S. (2004) Modulation of memory processes and cellular excitability in the dentate gyrus of freely moving rats by a 5-HT4 receptors partial agonist, and an antagonist. *Neuropharmacology*, 47, 1021-35.

MARSTON, H. M., REID, M. E., LAWRENCE, J. A., OLVERMAN, H. J. & BUTCHER, S. P. (1999) Behavioural analysis of the acute and chronic effects of MDMA treatment in the rat. *Psychopharmacology (Berl)*, 144, 67-76.

MAS, M., FARRE, M., DE LA, T. R., ROSET, P. N., ORTUNO, J., SEGURA, J. & CAMI, J. (1999) Cardiovascular and neuroendocrine effects and pharmacokinetics of 3, 4-methylenedioxymethamphetamine in humans. *J. Pharmacol. Exp. Ther.*, 290, 136-145.

MATSUMOTO, M., TOGASHI, H., MORI, K., UENO, K., OHASHI, S., KOJIMA, T. & YOSHIOKA, M. (2001) Evidence for involvement of central 5-HT(4) receptors in cholinergic function associated with cognitive processes: behavioral, electrophysiological, and neurochemical studies. *J. Pharmacol. Exp. Ther.*, 296, 676-82.

MCCANN, U. D., ELIGULASHVILI, V., MERTL, M., MURPHY, D. L. & RICAURTE, G. A. (1999a) Altered neuroendocrine and behavioral responses to m-chlorophenylpiperazine in 3,4-methylenedioxymethamphetamine (MDMA) users. *Psychopharmacology (Berl)*, 147, 56-65.

MCCANN, U. D., MERTL, M., ELIGULASHVILI, V. & RICAURTE, G. A. (1999b) Cognitive performance in (+/-) 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") users: a controlled study. *Psychopharmacology (Berl)*, 143, 417-425.

MCCANN, U. D. & RICAURTE, G. A. (1991) Lasting neuropsychiatric sequelae of (+-)methylenedioxymethamphetamine ('ecstasy') in recreational users. *J. Clin. Psychopharmacol.*, 11, 302-305.

MCCANN, U. D. & RICAURTE, G. A. (1992) MDMA ("ecstasy") and panic disorder: induction by a single dose. *Biol. Psychiatry*, 32, 950-953.

MCCANN, U. D., RIDENOUR, A., SHAHAM, Y. & RICAURTE, G. A. (1994) Serotonin neurotoxicity after (+/-)3,4-methylenedioxymethamphetamine (MDMA; "Ecstasy"): a controlled study in humans. *Neuropsychopharmacology*, 10, 129-138.

MCCANN, U. D., SLATE, S. O. & RICAURTE, G. A. (1996) Adverse reactions with 3,4-methylenedioxymethamphetamine (MDMA; 'ecstasy'). *Drug Saf*, 15, 107-115.

MCCANN, U. D., SZABO, Z., SCHEFFEL, U., DANNALS, R. F. & RICAURTE, G. A. (1998) Positron emission tomographic evidence of toxic effect of MDMA ("Ecstasy") on brain serotonin neurons in human beings. *Lancet*, 352, 1433-1437.

MCCANN, U. D., SZABO, Z., SECKIN, E., ROSENBLATT, P., MATHEWS, W. B., RAVERT, H. T., DANNALS, R. F. & RICAURTE, G. A. (2005) Quantitative PET studies of the serotonin transporter in MDMA users and controls using [11C]McN5652 and [11C]DASB. *Neuropsychopharmacology*, 30, 1741-1750.

MCCARDLE, K., LUEBBERS, S., CARTER, J. D., CROFT, R. J. & STOUGH, C. (2004) Chronic MDMA (ecstasy) use, cognition and mood. *Psychopharmacology (Berl)*, 173, 434-439.

MCCREARY, A. C., BANKSON, M. G. & CUNNINGHAM, K. A. (1999) Pharmacological studies of the acute and chronic effects of (+)-3, 4-methylenedioxymethamphetamine on locomotor activity: role of 5-hydroxytryptamine(1A) and 5-hydroxytryptamine(1B/1D) receptors. *J. Pharmacol. Exp. Ther.*, 290, 965-973.

MCDAID, J. & DOCHERTY, J. R. (2001) Vascular actions of MDMA involve alpha1 and alpha2-adrenoceptors in the anaesthetized rat. *Br. J. Pharmacol.*, 133, 429-437.

MCGREGOR, I. S., GURTMAN, C. G., MORLEY, K. C., CLEMENS, K. J., BLOKLAND, A., LI, K. M., CORNISH, J. L. & HUNT, G. E. (2003) Increased anxiety and "depressive" symptoms months after MDMA ("ecstasy") in rats: drug-induced hyperthermia does not predict long-term outcomes. *Psychopharmacology*, 168, 465-74.

MCGUIRE, P. (2000) Long term psychiatric and cognitive effects of MDMA use. *Toxicology Letters*, 112-113, 153-6.

MCTAVISH, S. F., COWEN, P. J. & SHARP, T. (1999) Effect of a tyrosine-free amino acid mixture on regional brain catecholamine synthesis and release. *Psychopharmacology (Berl)*, 141, 182-188.

MECHAN, A. O., ESTEBAN, B., O'SHEA, E., ELLIOTT, J. M., COLADO, M. I. & GREEN, A. R. (2002a) The pharmacology of the acute hyperthermic response that follows administration of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') to rats. *Br. J. Pharmacol.*, 135, 170-180.

MECHAN, A. O., MORAN, P. M., ELLIOTT, M., YOUNG, A. J., JOSEPH, M. H. & GREEN, R. (2002b) A study of the effect of a single neurotoxic dose of 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy") on the subsequent long-term behaviour of rats in the plus maze and open field. *Psychopharmacology(Berl)*, 159, 167-175.

MEHTA, M. A., MANES, F. F., MAGNOLFI, G., SAHAKIAN, B. J. & ROBBINS, T. W. (2004) Impaired set-shifting and dissociable effects on tests of spatial working memory following the dopamine D2 receptor antagonist sulpiride in human volunteers. *Psychopharmacology (Berl)*, 176, 331-42.

MENESES, A. (2003) A pharmacological analysis of an associative learning task: 5-HT1 to 5-HT7 receptor subtypes function on a Pavlovian/instrumental autoshaped memory. *Learning & Memory*, 10, 363-372.

MENESES, A. (2007) Do serotonin(1-7) receptors modulate short and long-term memory? *Neurobiol. Learn. Mem.*, 87, 561-72.

MENESES, A. & PEREZ-GARCIA, G. (2007) 5-HT1A receptors and memory. *Neurosci. Biobehav. Rev.*, 31, 705-727.

MERILL, J. (1996) Ecstasy and neurodegeneration. Advice is that "less is more". *Br. Med. J.*, 313, 423.

MICALE, V., LEGGIO, G. M., MAZZOLA, C. & DRAGO, F. (2006) Cognitive effects of SL65.0155, a serotonin 5-HT4 receptor partial agonist, in animal models of amnesia. *Brain Res.*, 1121, 207-15.

MILLER, R. T., LAU, S. S. & MONKS, T. J. (1997) 2,5-Bis-(glutathion-S-yl)-alpha-methyldopamine, a putative metabolite of (+/-)-3,4-methylenedioxyamphetamine, decreases brain serotonin concentrations. *Eur. J. Pharmacol.*, 323, 173-180.

MILNER, B., SQUIRE, L. R. & KANDEL, E. R. (1998) Cognitive neuroscience and the study of memory. Neuron, 20, 445-468.

MISSALE, C., NASH, S. R., ROBINSON, S. W., JABER, M. & CARON, M. G. (1998) Dopamine receptors: from structure to function. *Physiological Reviews*, 78, 189-225.

MITCHELL, E. S., HOPLIGHT, B. J., LEAR, S. P. & NEUMAIER, J. F. (2006) BGC20-761, a novel tryptamine analog, enhances memory consolidation and reverses scopolamine-induced memory deficit in social and visuospatial memory tasks through a 5-HT6 receptor-mediated mechanism. *Neuropharmacology*, 50, 412-20.

MONTGOMERY, C., FISK, J. E. & NEWCOMBE, R. (2005) The nature of ecstasy-group related deficits in associative learning. *Psychopharmacology (Berl)*, 180, 141-149.

MORGAN, M. J. (1998) Recreational use of "ecstasy" (MDMA) is associated with elevated impulsivity. *Neuropsychopharmacology*, 19, 252-264.

MORGAN, M. J. (1999) Memory deficits associated with recreational use of "ecstasy" (MDMA). *Psychopharmacology (Berl)*, 141, 30-36.

MORGAN, M. J. (2000) Ecstasy (MDMA): a review of its possible persistent psychological effects. *Psychopharmacology*, 152, 230-48.

MORGAN, M. J., MCFIE, L., FLEETWOOD, H. & ROBINSON, J. A. (2002) Ecstasy (MDMA): are the psychological problems associated with its use reversed by prolonged abstinence? *Psychopharmacology (Berl)*, 159, 294-303.

MORLEY, K. C., GALLATE, J. E., HUNT, G. E., MALLET, P. E. & MCGREGOR, I. S. (2001) Increased anxiety and impaired memory in rats 3 months after administration of 3,4-methylenedioxymethamphetamine ("ecstasy"). *Eur. J. Pharmacol.*, 433, 91-99.

MORLEY, K. C. & MCGREGOR, I. S. (2000) (+/-)-3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy') increases social interaction in rats. *Eur. J. Pharmacol.*, 408, 41-49.

MORTON, A. J., M.A., H. & L.C., D. (2001) Methamphetamine toxicity in mice is potentiated by exposure to loud music. *Neuroreport*, 12, 3277-3281.

MUMBY, D. G. (2001) Perspectives on object-recognition memory following hippocampal damage: lessons from studies in rats. *Behav. Brain Res.*, 127, 159-181.

NAIR, S. G. & GUDELSKY, G. A. (2005) 3,4-Methylenedioxymethamphetamine (MDMA) enhances the release of acetylcholine by 5-HT4 and D1 receptor mechanisms in the rat prefrontal cortex. *Synapse*, 58, 229-235.

NAIR, S. G. & GUDELSKY, G. A. (2006) 3,4-Methylenedioxymethamphetamine enhances the release of acetylcholine in the prefrontal cortex and dorsal hippocampus of the rat. *Psychopharmacology*, 184, 182-9.

NASH, J. F. (1990) Ketanserin pretreatment attenuates MDMA-induced dopamine release in the striatum as measured by in vivo microdialysis. *Life Sci.*, 47, 2401-2408.

NASH, J. F. & BRODKIN, J. (1991) Microdialysis studies on 3,4-methylenedioxymethamphetamine-induced dopamine release: effect of dopamine uptake inhibitors. *J. Pharmacol. Exp. Ther.*, 259, 820-825.

NASH, J. F. & YAMAMOTO, B. K. (1992) Methamphetamine neurotoxicity and striatal glutamate release: comparison to 3,4-methylenedioxymethamphetamine. *Brain Res.*, 581, 237-243.

NAVARRO, J. F. & MALDONADO, E. (2002) Acute and subchronic effects of MDMA ("ecstasy") on anxiety in male mice tested in the elevated plus-maze. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 26, 1151-1154.

NESTLER, E. J., HYMAN, S. E. & MALENKA, R. C. (2001) *Molecular Neuropharmacology: A Foundation for Clinical Neuroscience*, London, The McGraw-Hill Companies Medical Publishing Division.

NICHOLS, D. E. (1986) Differences between the mechanism of action of MDMA, MBDB, and the classic hallucinogens. Identification of a new therapeutic class: entactogens. *J. Psychoactive Drugs*, 18, 305-313.

NUTT, D. J. (2009) Equasy-An overlooked addiction with implications for the current debate on drug harms. *J Psychopharmacol,* 23, 3-5.

O'CALLAGHAN, J. P. & MILLER, D. B. (1994) Neurotoxicity profiles of substituted amphetamines in the C57BL/6J mouse. *J. Pharmacol. Exp. Ther.*, 270, 741-751.

O'HEARN, E., BATTAGLIA, G., DE SOUZA, E. B., KUHAR, M. J. & MOLLIVER, M. E. (1988) Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence for neurotoxicity. *J. Neurosci.*, 8, 2788-2803.

O'SHEA, E., ESTEBAN, B., CAMARERO, J., GREEN, A. R. & COLADO, M. I. (2001) Effect of GBR 12909 and fluoxetine on the acute and long term changes induced by MDMA ('ecstasy') on the 5-HT and dopamine concentrations in mouse brain. *Neuropharmacology*, 40, 65-74.

O'SHEA, E., GRANADOS, R., ESTEBAN, B., COLADO, M. I. & GREEN, A. R. (1998) The relationship between the degree of neurodegeneration of rat brain 5-HT nerve terminals and the dose and frequency of administration of MDMA ('ecstasy'). *Neuropharmacology*, 37, 919-926.

O'SHEA, E., ORIO, L., ESCOBEDO, I., SANCHEZ, V., CAMARERO, J., GREEN, A. R. & COLADO, M. I. (2006) MDMA-induced neurotoxicity: long-term effects on 5-HT biosynthesis and the influence of ambient temperature. *Br. J. Pharmacol.*, 148, 778-85.

OAK, J. N., OLDENHOF, J. & VAN TOL, H. H. M. (2000) The dopamine D4 receptor: one decade of research. *Eur. J. Pharmacol.*, 405, 303-327.

OGREN, S. O., ERIKSSON, T., M., ELVANDER-TOTTIE, E., D'ADDARIO, C., EKSTRÖM, J. C., SVENNINGSSON, P., MEISTER, B., KEHR, J. & STIEDL, O. (2008) The role of 5-HT(1A) receptors in learning and memory. *Behav. Brain Res.*, 195, 54-77.

PARIS, J. M. & CUNNINGHAM, K. A. (1992) Lack of serotonin neurotoxicity after intraraphe microinjection of (+)-3,4-methylenedioxymethamphetamine (MDMA). *Brain Res. Bull.*, 28, 115-119.

PARROTT, A. C. (2001) Human psychopharmacology of Ecstasy (MDMA): a review of 15 years of empirical research. *Hum. Psychopharmacol.*, 16, 557-577.

PARROTT, A. C. (2002) Recreational Ecstasy/MDMA, the serotonin syndrome, and serotonergic neurotoxicity. *Pharmacol. Biochem.Behav.*, 71, 837-44.

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

PARROTT, A. C. (2005) Chronic tolerance to recreational MDMA (3,4-methylenedioxymethamphetamine) or Ecstasy. *J. Psychopharmacol.*, 19, 71-83.

PARROTT, A. C. (2006) MDMA in humans: factors which affect the neuropsychobiological profiles of recreational ecstasy users, the integrative role of bioenergetic stress. *J. Psychopharmacol.*, 20, 147-163.

PARROTT, A. C. & LASKY, J. (1998) Ecstasy (MDMA) effects upon mood and cognition: before, during and after a Saturday night dance. *Psychopharmacology (Berl)*, 139, 261-268.

PARROTT, A. C., SISK, E. & TURNER, J. J. (2000) Psychobiological problems in heavy 'ecstasy' (MDMA) polydrug users. *Drug Alcohol Depend.*, 60, 105-110.

PARTILLA, J. S., DEMPSEY, A. G., NAGPAL, A. S., BLOUGH, B. E., BAUMANN, M. H. & ROTHMAN, R. B. (2006) Interaction of amphetamines and related compounds at the vesicular monoamine transporter. *J. Pharmacol. Exp. Ther.*, 319, 237-246.

PAXINOS, G. & WATSON, C. (1998) *The Rat Brain in Stereotaxis Coordinates, 4th edition.*, London, Academic Press.

PÉREZ-GARCÍA, G., GONZALEZ-ESPINOSA, C. & MENESES, A. (2006) An mRNA expression analysis of stimulation and blockade of 5-HT7 receptors during memory consolidation. *Behav. Brain Res.*, 169, 83-92.

PEREZ-GARCÍA, G. & MENESES, A. (2005a) Oral administration of the 5-HT6 receptor antagonists SB-357134 and SB-399885 improves memory formation in an autoshaping learning task. *Pharmacol. Biochem. Behav.*, 81, 673-82.

PEREZ-GARCÍA, G. S. & MENESES, A. (2005b) Effects of the potential 5-HT7 receptor agonist AS 19 in an autoshaping learning task. *Behav. Brain Res.*, 163, 136-40.

PEROUTKA, S. J. (1987) Incidence of recreational use of 3,4-methylenedimethoxymethamphetamine (MDMA, "ecstasy") on an undergraduate campus. *N. Engl. J. Med.*, 317, 1542-1543.

PIPER, B. J. (2007) A developmental comparison of the neurobehavioral effects of ecstasy (MDMA). *Neurotoxicology & Teratology*, 29, 288-300.

PIPER, B. J. & MEYER, J. S. (2004) Memory deficit and reduced anxiety in young adult rats given repeated intermittent MDMA treatment during the periadolescent period. *Pharmacol. Biochem. Behav.*, 79, 723-31.

PITSIKAS, N., RIGAMONTI, A. E., CELLA, S. G. & MULLER, E. E. (2003) The 5-HT1A receptor antagonist WAY 100635 improves rats performance in different models of amnesia evaluated by the object recognition task.. *Brain Res.*, 983, 215-222.

PITSIKAS, N., TSITSIRIGOU, S., ZISOPOULOU, S. & SAKELLARIDIS, N. (2005) The 5-HT1A receptor and recognition memory: Possible modulation of its behavioral effects by the nitrergic system. *Behav. Brain Res.*, 159, 287-293.

PIZARRO, N., FARRE, M., PUJADAS, M., PEIRO, A. M., ROSET, P. N., JOGLAR, J. & DE LA, T. R. (2004) Stereochemical analysis of 3,4-methylenedioxymethamphetamine and its main metabolites in human samples including the catechol-type metabolite (3,4-dihydroxymethamphetamine). *Drug Metabolism & Disposition*, 32, 1001-7.

POMPEI, P., CAVAZZUTI, E., MARTARELLI, D., PEDICONI, D., ARLETTI, R., LUCAS, L. & MASSI, M. (2002) Preprotachykinin A gene expression after administration of 3,4-methylene dioxymethamphetamine (Ecstasy). *Eur. J. Pharmacol.*, 450, 245-251.

RENEMAN, L., BOOIJ, J., DE, B. K., REITSMA, J. B., DE WOLFF, F. A., GUNNING, W. B., DEN HEETEN, G. J. & VAN DEN, B. W. (2001a) Effects of dose, sex, and long-term abstention from use on toxic effects of MDMA (ecstasy) on brain serotonin neurons. *Lancet*, 358, 1864-1869.

RENEMAN, L., ENDERT, E., DE, B. K., LAVALAYE, J., FEENSTRA, M. G., DE WOLFF, F. A. & BOOIJ, J. (2002) The acute and chronic effects of MDMA ("ecstasy") on cortical 5-HT2A receptors in rat and human brain. *Neuropsychopharmacology*, 26, 387-96.

RENEMAN, L., LAVALAYE, J., SCHMAND, B., DE WOLFF, F. A., VAN DEN, B. W., DEN HEETEN, G. J. & BOOIJ, J. (2001b) Cortical serotonin transporter density and verbal memory in individuals who stopped using 3,4-

methylenedioxymethamphetamine (MDMA or "ecstasy"): preliminary findings. *Archives of General Psychiatry*, 58, 901-6.

RICAURTE, G. A., DELANNEY, L. E., IRWIN, I. & LANGSTON, J. W. (1988) Toxic effects of MDMA on central serotonergic neurons in the primate: importance of route and frequency of drug administration. *Brain Res.*, 446, 165-168.

RICAURTE, G. A., MARTELLO, A. L., KATZ, J. L. & MARTELLO, M. B. (1992) Lasting effects of (+-)-3,4-methylenedioxymethamphetamine (MDMA) on central serotonergic neurons in nonhuman primates: neurochemical observations. *J. Pharmacol. Exp. Ther.*, 261, 616-622.

RIEDEL, G., PLATT, B. & MICHEAU, J. (2003) Glutamate receptor function in learning and memory. *Brain Res.* 140, 1-47.

ROGERS, R. D. & ROBBINS, T. W. (2001) Investigating the neurocognitive deficits associated with chronic drug misuse. *Curr. Opin. Neurobiol.*, 11, 250-257.

ROTHMAN, R. B., BAUMANN, M. H., DERSCH, C. M., ROMERO, D. V., RICE, K. C., CARROLL, F. I. & PARTILLA, J. S. (2001) Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. *Synapse*, 39, 32-41.

SAADAT, K. S., ELLIOTT, J. M., COLADO, M. I. & GREEN, A. R. (2004) Hyperthermic and neurotoxic effect of 3,4-methylenedioxymethamphetamine (MDMA) in guinea pigs. *Psychopharmacology*, 173, 452-3.

SABOL, K. E., LEW, R., RICHARDS, J. B., VOSMER, G. L. & SEIDEN, L. S. (1996) Methylenedioxymethamphetamine-induced serotonin deficits are followed by partial recovery over a 52-week period. Part I: Synaptosomal uptake and tissue concentrations. *J. Pharmacol. Exp. Ther.*, 276, 846-854.

SALDANA, S. N. & BARKER, E. L. (2004) Temperature and 3,4-methylenedioxymethamphetamine alter human serotonin transporter-mediated dopamine uptake. *Neurosci.Lett.*, 354, 209-212.

SAMBETH, A., BLOKLAND, A., HARMER, C. J., KILKENS, T. O., NATHAN, P. J., PORTER, R. J., SCHMITT, J. A., SCHOLTISSEN, B., SOBCZAK, S., YOUNG, A. H. & RIEDEL, W. J. (2007) Sex differences in the effect of acute tryptophan depletion on declarative episodic memory: a pooled analysis of nine studies. *Neurosci. Biobehav. Rev.*, 31, 516-29.

SANCHEZ, V., CAMARERO, J., ESTEBAN, B., PETER, M. J., GREEN, A. R. & COLADO, M. I. (2001) The mechanisms involved in the long-lasting neuroprotective effect of fluoxetine against MDMA ('ecstasy')-induced degeneration of 5-HT nerve endings in rat brain. *Br. J. Pharmacol.*, 134, 46-57.

SANCHEZ, V., O'SHEA, E., SAADAT, K. S., ELLIOTT, J. M., COLADO, M. I. & GREEN, A. R. (2004) Effect of repeated ('binge') dosing of MDMA to rats housed at normal and high temperature on neurotoxic damage to cerebral 5-HT and dopamine neurones. *J. Psychopharmacol.*, 18, 412-416.

SAWAGUCHI, T. & GOLDMAN-RAKIC, P. S. (1991) D1 dopamine receptors in prefrontal cortex: involvement in working memory. *Science*, 251, 947-950.

SAWAGUCHI, T. & GOLDMAN-RAKIC, P. S. (1994) The role of D1-dopamine receptor in working memory: local injections of dopamine antagonists into the prefrontal cortex of rhesus monkeys performing an oculomotor delayed-response task. *J. Neurophysiol.*, 71, 515-528.

SCANZELLO, C. R., HATZIDIMITRIOU, G., MARTELLO, A. L., KATZ, J. L. & RICAURTE, G. A. (1993) Serotonergic recovery after (+/-)3,4-(methylenedioxy) methamphetamine injury: observations in rats. *J. Pharmacol. Exp. Ther.*, 264, 1484-1491.

SCHIAPPARELLI, L., SIMON, A. M., DEL RIO, J. & FRECHILLA, D. (2006) Opposing effects of AMPA and 5-HT1A receptor blockade on passive avoidance and object recognition performance, correlation with AMPA receptor subunit expression in rat hippocampus. *Neuropharmacology*, 50, 897-907.

SCHIFANO, F. (1991) Chronic atypical psychosis associated with MDMA ("ecstasy") abuse. *Lancet*, 338, 1335.

SCHIFANO, F., DI, F. L., FORZA, G., MINICUCI, N. & BRICOLO, R. (1998) MDMA ('ecstasy') consumption in the context of polydrug abuse: a report on 150 patients. *Drug Alcohol Depend.*, 52, 85-90.

SCHIFANO, F., OYEFESO, A., CORKERY, J., COBAIN, K., JAMBERT-GRAY, R., MARTINOTTI, G. & GHODSE, A. H. (2003) Death rates from ecstasy (MDMA, MDA) and polydrug use in England and Wales 1996-2002. *Hum. Psychopharmacol.*, 18, 519-524.

SCHMIDT, C. J. (1987) Acute administration of methylenedioxymethamphetamine: comparison with the neurochemical effects of its N-desmethyl and N-ethyl analogs. *Eur. J. Pharmacol.*, 136, 81-88.

SCHMIDT, C. J., BLACK, C. K., ABBATE, G. M. & TAYLOR, V. L. (1990) Methylenedioxymethamphetamine-induced hyperthermia and neurotoxicity are independently mediated by 5-HT2 receptors. *Brain Res.*, 529, 85-90.

SCHMIDT, C. J., LEVIN, J. A. & LOVENBERG, W. (1987) In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain. *Biochem.Pharmacol.*, 36, 747-755.

SCHMIDT, C. J., SULLIVAN, C. K. & FADAYEL, G. M. (1994) Blockade of striatal 5-hydroxytryptamine2 receptors reduces the increase in extracellular concentrations of dopamine produced by the amphetamine analogue 3,4-methylenedioxymethamphetamine. *J. Neurochem.*, 62, 1382-1389.

SCHMIDT, C. J. & TAYLOR, V. L. (1988) Direct central effects of acute methylenedioxymethamphetamine on serotonergic neurons. *Eur. J. Pharmacol.*, 156, 121-131.

SCHMIDT, C. J., TAYLOR, V. L., ABBATE, G. M. & NIEDUZAK, T. R. (1991) 5-HT2 antagonists stereoselectively prevent the neurotoxicity of 3,4-methylenedioxymethamphetamine by blocking the acute stimulation of dopamine synthesis: reversal by L-dopa. *J. Pharmacol. Exp. Ther.*, 256, 230-235.

SCHOLEY, A. B., PARROTT, A. C., BUCHANAN, T., HEFFERNAN, T. M., LING, J. & RODGERS, J. (2004) Increased intensity of Ecstasy and polydrug usage in the

more experienced recreational Ecstasy/MDMA users: a WWW study. *Addictive Behaviors*, 29, 743-52.

SEGAL, D. S. & KUCZENSKI, R. (1994) Behavioral pharmacology of amphetamine. IN CHO, A. & SEGAL, D. S. (Eds.) *Amphetamine and its analogs*. San Diego, CA, Academic.

SEGURA, M., ORTUNO, J., FARRE, M., MCLURE, J. A., PUJADAS, M., PIZARRO, N., LLEBARIA, A., JOGLAR, J., ROSET, P. N., SEGURA, J. & DE LA, T. R. (2001) 3,4-Dihydroxymethamphetamine (HHMA). A major in vivo 3,4-methylenedioxymethamphetamine (MDMA) metabolite in humans. Chem. *Res. Toxicol.*, 14, 1203-1208.

SEIDEN, L. S., SABOL, K. E. & RICAURTE, G. A. (1993) Amphetamine: effects on catecholamine systems and behavior. *Annu. Rev. Pharmacol. Toxicol.*, 33, 639-677.

SEMPLE, D. M., EBMEIER, K. P., GLABUS, M. F., O'CARROLL, R. E. & JOHNSTONE, E. C. (1999) Reduced in vivo binding to the serotonin transporter in the cerebral cortex of MDMA ('ecstasy') users. *Br. J. Psychiatry*, 175, 63-69.

SHANKARAN, M. & GUDELSKY, G. A. (1998) Effect of 3,4-methylenedioxymethamphetamine (MDMA) on hippocampal dopamine and serotonin. *Pharmacol. Biochem. Behav.*, 61, 361-366.

SHANKARAN, M. & GUDELSKY, G. A. (1999) A neurotoxic regimen of MDMA suppresses behavioral, thermal and neurochemical responses to subsequent MDMA administration. *Psychopharmacology (Berl)*, 147, 66-72.

SHANKARAN, M., YAMAMOTO, B. K. & GUDELSKY, G. A. (1999) Mazindol attenuates the 3,4-methylenedioxymethamphetamine-induced formation of hydroxyl radicals and long-term depletion of serotonin in the striatum. *J. Neurochem.*, 72, 2516-2522.

SHANKARAN, M., YAMAMOTO, B. K. & GUDELSKY, G. A. (2001) Ascorbic acid prevents 3,4-methylenedioxymethamphetamine (MDMA)-induced hydroxyl radical formation and the behavioral and neurochemical consequences of the depletion of brain 5-HT. *Synapse*, 40, 55-64.

SHIODA, K., NISIJIMA, K., YOSHINO, T., KUBOSHIMA, K., IWAMURA, T., YUI, K. & KATO, S. (2008) Risperidone attenuates and reverses hyperthermia induced by 3,4-methylenedioxymethamphetamine (MDMA) in rats. *Neurotoxicology*, 29, 1030-1036.

SHULGIN, A. T. (1986) The background and chemistry of MDMA. *J. Psychoactive Drugs*, 18, 291-304.

SKELTON, M. R., ABLE, J. A., GRACE, C. E., HERRING, N. R., SCHAEFER, T. L., GUDELSKY, G. A., VORHEES, C. V. & WILLIAMS, M. T. (2008) (+/-)-3,4-Methylenedioxymethamphetamine treatment in adult rats impairs path integration learning: a comparison of single vs once per week treatment for 5 weeks. *Neuropharmacology*, 55, 1121-30.

SLIKKER, W. J., ALI, S. F., SCALLET, A. C., FRITH, C. H., NEWPORT, G. D. & BAILEY, J. R. (1988) Neurochemical and neurohistological alterations in the rat and monkey produced by orally administered methylenedioxymethamphetamine (MDMA). *Toxicol. Appl. Pharmacol.*, 94, 448-457.

SOAR, K., TURNER, J. J. & PARROTT, A. C. (2006) Problematic versus non-problematic ecstasy/MDMA use: the influence of drug usage patterns and pre-existing psychiatric factors. *J.Psychopharmacol.*, 20, 417-424.

SOKOLOFF, P. & SCHWARTZ, J. C. (1995) Novel dopamine receptors half a decade later. Trends Pharmacol Sci., 16, 270-275.

SPANO, P. F., GOVONI, S. & TRABUCCHI, M. (1978) Studies on the pharmacological properties of dopamine receptors in various areas of the Central Nervous System. *Adv. Biochem. Psychopharmacol.*, 29, 155-165.

SPANOS, L. J. & YAMAMOTO, B. K. (1989) Acute and subchronic effects of methylenedioxymethamphetamine [(+/-)MDMA] on locomotion and serotonin syndrome behavior in the rat. *Pharmacol. Biochem. Behav.*, 32, 835-840.

SPRAGUE, J. E., EVERMAN, S. L. & NICHOLS, D. E. (1998) An integrated hypothesis for the serotonergic axonal loss induced by 3,4-methylenedioxymethamphetamine. *Neurotoxicology*, 19, 427-441.

SPRAGUE, J. E. & NICHOLS, D. E. (1995) The monoamine oxidase-B inhibitor L-deprenyl protects against 3,4-methylenedioxymethamphetamine-induced lipid peroxidation and long-term serotonergic deficits. *J. Pharmacol. Exp. Ther.*, 273, 667-673.

SPRAGUE, J. E., PRESTON, A. S., LEIFHEIT, M. & WOODSIDE, B. (2003) Hippocampal serotonergic damage induced by MDMA (ecstasy): effects on spatial learning. *Physiol. Behav.*, 79, 281-287.

SQUIRE, L. R., STARK, C. E. & CLARK, R. E. (2004) The medial temporal lobe. Annual Reviews in the Neurosciences, 27, 279-306.

STANLEY, N., SALEM, A. & IRVINE, R. J. (2007) The effects of co-administration of 3,4-methylenedioxymethamphetamine ("ecstasy") or para-methoxyamphetamine and moclobemide at elevated ambient temperatures on striatal 5-HT, body temperature and behavior in rats. *Neuroscience,* 146, 321-329.

STONE, D. M., JOHNSON, M., HANSON, G. R. & GIBB, J. W. (1987a) A comparison of the neurotoxic potential of methylenedioxyamphetamine (MDA) and its N-methylated and N-ethylated derivatives. *Eur. J. Pharmacol.*, 134, 245-248.

STONE, D. M., JOHNSON, M., HANSON, G. R. & GIBB, J. W. (1988) Role of endogenous dopamine in the central serotonergic deficits induced by 3,4-methylenedioxymethamphetamine. *J. Pharmacol. Exp. Ther.*, 247, 79-87.

STONE, D. M., MERCHANT, K. M., HANSON, G. R. & GIBB, J. W. (1987b) Immediate and long-term effects of 3,4-methylenedioxymethamphetamine on serotonin pathways in brain of rat. *Neuropharmacology*, 26, 1677-1683.

STONE, D. M., STAHL, D. C., HANSON, G. R. & GIBB, J. W. (1986) The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) on monoaminergic systems in the rat brain. *Eur. J. Pharmacol.*, 128, 41-48.

SUMNALL, H. R., O'SHEA, E., MARSDEN, C. A. & COLE, J. C. (2004) The effects of MDMA pretreatment on the behavioural effects of other drugs of abuse in the rat elevated plus-maze test. Pharmacol.Biochem.Behav., 77, 805-814.

SUZUKI, W. A. & CLAYTON, N. S. (2000) The hippocampus and memory: a comparative and ethological perspective. *Curr.Opin.Neurobiol.*, 10, 768-73.

TAFFE, M. A., WEED, M. R., DAVIS, S., HUITRON-RESENDIZ, S., SCHROEDER, R., PARSONS, L. H., HENRIKSEN, S. J. & GOLD, L. H. (2001) Functional consequences of repeated (+/-)3,4-methylenedioxymethamphetamine (MDMA) treatment in rhesus monkeys. *Neuropsychopharmacology*, 24, 230-239.

TERRY, A. V. J., BUCCAFUSCO, J. J., JACKSON, W. J., PRENDERGAST, M. A., FONTANA, D. J., WONG, E. H., BONHAUS, D. W., WELLER, P. & EGLEN, R. M. (1998) Enhanced delayed matching performance in younger and older macaques administered the 5-HT4 receptor agonist, RS 17017. *Psychopharmacology (Berl)*, 135, 407-15.

TEUCHERT-NOODT, G., DAWIRS, R. R. & HILDEBRANDT, K. (2000) Adult treatment with methamphetamine transiently decreases dentate granule cell proliferation in the gerbil hippocampus. *J. Neural. Transm.*, 107, 133-143.

TOPP, L., HANDO, J., DILLON, P., ROCHE, A. & SOLOWIJ, N. (1999) Ecstasy use in Australia: patterns of use and associated harm. *Drug Alcohol Depend.*, 55, 105-115.

UCHIDA, S., UMEEDA, H., KITAMOTO, A., MASUSHIGE, S. & KIDA, S. (2007) Chronic reduction in dietary tryptophan leads to a selective impairment of contextual fear memory in mice. *Brain Res.*, 1149, 149-56.

UNDOC (2008) *World Drug Report*, Vienna, United Nations Office on Drugs and Crime.

VAKALOPOULOS, C. (2006) Neuropharmacology of cognition and memory: A unify theory of neuromodulator imbalance in psychiatry and amnesia. *Medical Hypotheses*, 66, 394-431.

VERKES, R. J., GIJSMAN, H. J., PIETERS, M. S., SCHOEMAKER, R. C., DE, V. S., KUIJPERS, M., PENNINGS, E. J., DE, B. D., VAN DE, W. G., VAN GERVEN, J. M. & COHEN, A. F. (2001) Cognitive performance and serotonergic function in users of ecstasy. *Psychopharmacology (Berl)*, 153, 196-202.

VERTES, R. P. (1991) A Pha-L analysis of ascending projections of the dorsal raphe nucleus in the rat. *J. Comp. Neurol.*, 313, 643-68.

VERTES, R. P., FORTIN, W. J. & CRANE, A. M. (1999) Projections of the median raphe nucleus in the rat. *J. Comp. Neurol.*, 407, 555-82.

VOLLENWEIDER, F. X., GAMMA, A., LIECHTI, M. & HUBER, T. (1998) Psychological and cardiovascular effects and short-term sequelae of MDMA ("ecstasy") in MDMA-naive healthy volunteers. *Neuropsychopharmacology*, 19, 241-251.

VON GEUSAU, N. A., STALENHOEF, P., HUIZINGA, M., SNEL, J. & RIDDERINKHOF, K. R. (2004) Impaired executive function in male MDMA ("ecstasy") users. *Psychopharmacology (Berl)*, 175, 331-341.

VON HUBEN, S. N., DAVIS, S. A., LAY, C. C., KATNER, S. N., CREAN, R. D. & TAFFE, M. A. (2006) Differential contributions of dopaminergic D1- and D2-like receptors to cognitive function in rhesus monkeys. *Psychopharmacology (Berl)*, 188, 586-96.

WARBURTON, E. C., HARRISON, A. A., ROBBINS, T. W. & EVERITT, B. J. (1997) Contrasting effects of systemic and intracerebral infusions of the 5-HT1A receptor agonist 8-OH-DPAT on spatial short-term working memory in rats. *Behav. Brain Res.*, 84, 247-58.

WAREING, M., FISK, J. E. & MURPHY, P. N. (2000) Working memory deficits in current and previous users of MDMA ('ecstasy'). *Br. J. Psychol.*, 91 181-188.

WILLIAMS, M. T., MORFORD, L. L., WOOD, S. L., ROCK, S. L., MCCREA, A. E., FUKUMURA, M., WALLACE, T. L., BROENING, H. W., MORAN, M. S. & VORHEES, C. V. (2003) Developmental 3,4-methylenedioxymethamphetamine (MDMA) impairs sequential and spatial but not cued learning independent of growth, litter effects or injection stress. *Brain Res.*, 968, 89-101.

WINSTOCK, A. R., GRIFFITHS, P. & STEWART, D. (2001) Drugs and the dance music scene: a survey of current drug use patterns among a sample of dance music enthusiasts in the UK. *Drug Alcohol Depend.*, 64, 9-17.

WOOLLEY, M. L., BENTLEY, J. C., SLEIGHT, A. J., MARSDEN, C. A. & FONE, K. C. (2001) A role for 5-ht6 receptors in retention of spatial learning in the Morris water maze. *Neuropharmacology*, 41, 210-9.

WOOLLEY, M. L., MARSDEN, C. A., SLEIGHT, A. J. & FONE, K. C. (2003) Reversal of a cholinergic-induced deficit in a rodent model of recognition memory by the selective 5-HT6 receptor antagonist, Ro 04-6790. *Psychopharmacology (Berl)*, 170, 358-67.

WURTMANM, R. J. & MELAMED, E. (1981) Precusor control of neurotransmitter synthesis. *Pharmacol. Rev.*, 32, 315-335.

YAMAGUCHI, M., SUZUKI, T., SEKI, T., NAMBA, T., JUAN, R., ARAI, H., HORI, T. & ASADA, T. (2004) Repetitive cocaine administration decreases neurogenesis in adult rat hippocampus. *Ann.N.Y.Acad.Sci.*, 1025, 351-362.

YAMAMOTO, B. K., NASH, J. F. & GUDELSKY, G. A. (1995) Modulation of methylenedioxymethamphetamine-induced striatal dopamine release by the interaction between serotonin and gamma-aminobutyric acid in the substantia nigra. *J. Pharmacol. Exp. Ther.*, 273, 1063-1070.

YAMAMOTO, B. K. & SPANOS, L. J. (1988) The acute effects of methylenedioxymethamphetamine on dopamine release in the awake-behaving rat. *Eur. J. Pharmacol.*, 148, 195-203.

YUAN, J., CORD, B. J., MCCANN, U. D., CALLAHAN, B. T. & RICAURTE, G. A. (2002) Effect of glucoprivation on serotonin neurotoxicity induced by substituted amphetamines. *J. Pharmacol. Exp. Ther.*, 303, 831-839.

ZAHRT, J., TAYLOR, J. R., MATHEW, R. G. & ARNSTEN, A. F. (1997) Supranormal stimulation of D1 dopamine receptors in the rodent prefrontal cortex impairs working memory performance. *J. Neurosci.*, 17, 8528-35.

ZAKZANIS, K. K. & YOUNG, D. A. (2001) Executive function in abstinent MDMA ('ecstasy') users. *Med. Sci. Monit.*, 7, 1292-1298.

APPENDIX I

Solution preparation for analysis of brain tissue amines

(A) Mobile Phase (500 ml)

0.05 M	KH_2PO_4	3.4023 g
0.1 mM	EDTA	0.0186 g
0.16 mM	Sodium octanylsulfonate	0.0173 g
13.5 % v/v	Methanol	67.5 ml

Made up to 500 ml by HPLC grade water and adjust pH to 3.0 with *o*-phosphoric acid

(B) Perchloric acid (PCA) stock solution (1 M) (250 ml)

0.2 % w/v	Sodium metabisulfite	0.5 g
1 % w/v	EDTA	0.25 g
	PCA (60%)	21.6 ml

Made up to 250 ml by HPLC grade water

(C) Amines standard stock solution (10^{-2} M)

Dopamine	0.0095 g/5 ml
DOPAC	0.0084 g/5ml
HVA	0.0091 g/5 ml
5-HT	0.0194 g/5 ml
5-HIAA	0.0096 g/5 ml

Aliquot 100 µl into 1.5 ml eppendorf tubes and keep in - 80 °C freezer

APPENDIX II

Mobile phase for analysis of 5-HT in microdialysis samples

All chemicals used in HPLC analysis were of HPLC grade. pH was adjusted to 3.0 with *o*-phosphoric acid. The mobile phase was filtered with 45 mM millipore flask filter and sonicated for 15 min before using.

Table AI Chemical compositions of the mobile phase for HPLC

Compounds	Concentration	g/litre
KH_2PO_4	0.05 M	6.8046
EDTA	0.1 mM	0.0372
Sodium octanylsulfonate	120 mg/L	0.120
KCl	8 mM	0.5964
Methanol	15 %	150 ml

APPENDIX III

Artificial cerebrospinal Fluid Solution (aCSF)

All chemicals, except $CaCl_2$, were dissolved together with distilled millipore water. $CaCl_2$ was separately dissolved in distilled water before added to the aCSF solution. pH was adjusted to 7.4 with *o*-phosphoric acid. aCSF solution was filtered twice with 45 mM millipore flask filter and sonicated for 15 min before using.

Table AII Chemical compositions of the aCSF for *in vivo* microdialysis

Compounds	Concentration (mM)	g/litre
NaCl	125	7.30
$NaHCO_3$	27.0	2.268
KCl	2.5	0.186
NaH_2PO_4	0.5	0.069
Na_2HPO_4	1.2	0.32
$NaSO_4$	0.5	0.072
$MgCl_2$	1.0	0.204
$CaCl_2.2H_2O$	1.0	0.148

APPENDIX IV

Solution preparations for analysis of brain tissue tyrosine

For the measurement of tyrosine, HPLC with pre-column derivatisation coupled with electrochemical detection (ECD) was utilized. The HPLC-ECD method was modified from Bongiovanni et al (2001).

(A) Mobile phase

All chemicals used in HPLC analysis were of HPLC grade. pH was adjusted to 6.8 with *o*-phosphoric acid. The mobile phase was filtered with 45 mM millipore flask filter and sonicated for 15 min before using.

Table AIII Chemical compositions of the mobile phase for HPLC

Compounds	Concentration	g/litre
Na_2HPO_4	0.133 M	47.6326
EDTA	0.15 mM	0.0558
Methanol	20 %	200 ml

(B) Tyrosine stock solution (1 mg/ml)

1. Weighing Tyrosine HCl (Sigma) 10 mg into a 10-ml amber volumetric flask

2. Add 9.5 ml of diluent (H_2O : MeOH 75:50 v/v) and 0.5 ml of 30% w/v NaOH (3 mg NaOH + 10 ml H_2O) to make 10-ml of 1 mg/ml tyrosine stock solution

3. Aliquot 50 µl of the tyrosine stock solution into 0.5 ml amber eppendorf tube and keep in -80 °C freezer

(C) Derivatising agent (OPA-S)

1. Weighing *o*-phthaldehyde (OPA) 10 mg and 30 mg sodium sulfite into 50-ml volumetric flask

2. Add 0.25 ml water (HPLC grade) and 0.25 ml Methanol

3. Vortex-mixed

4. Add 4.5 ml Sodium borate buffer (0.4 M Boric acid pH 10.4 with 6 M NaOH)

0.4 M Boric acid (H_3BO_3, MW 61.83)	
Boric acid	2.4732 g in 100 ml H_2O
6 M NaOH (MW 40)	
NaOH	2.4 g in 10 ml H_2O

(D) Perchloric acid (0.1 M) containing 1.0 μg/ml norvaline

1. Make 1 M PCA by

60% Perchloric acid (PCA) 21.6 ml in HPLC grade water made up to 250 ml

2. Dilute 1 M PCA to 0.1 M PCA
3. Make 100 μg/ml norvaline in 0.1 M PCA by

1 mg norvaline + 10 ml of 0.1 M PCA

4. Dilute 100 μg/ml norvaline to 1 μg/ml norvaline using 0.1 M PCA

(E) Tissue sample preparation

1. Weighing brain tissue into 5-ml plastic tube
2. Add 1 ml of ice-cold 0.1 M PCA containing 1 μg/ml norvaline
3. Sonicate 20-30 s
4. Centrifuge at 16000 g 4 °C 4 min
5. Keep supernatant in -80 °C for later analysis

(F) Derivatisation

1. Pipette 100 μl of sample or standard into the 1.5-ml eppebdorf centrifuge tube
2. Add 100 μl derivatising agent (OPA-S) and 100 μl sodium borate buffer
3. Sample or standard react to OPA-S for 5 min at room temperature
4. Made the reaction samples or standard up to 1 ml with mobile phase and injected 20 μl onto column