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Investigation of cerebral perfusion changes following MDMA "Ecstasy" administration in an animal model using bolus-tracking arterial spin labelling MRI

by

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Thesis submitted for the degree of Doctor of Philosophy at the University of Dublin, Trinity College

Submitted October 2011

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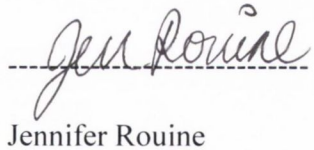

Jennifer Rouine

II Summary

The recreational drug of abuse 3,4-methylenedioxymethamphetamine (MDMA; Ecstasy) carries a risk of cerebrovascular accidents (CVA) that may relate to the role of serotonin (5-HT) and/or dopamine in the regulation of cerebrovascular tone. Recent advances in magnetic resonance imaging (MRI) have enabled measurement of cerebral blood perfusion using contrast agent-free approaches such as bolus-tracking arterial spin labeling (btASL). This investigation assessed changes in cerebral perfusion following systemic MDMA administration to rats using btASL MRI. Adult male Wistar rats were administered MDMA (5 or 20 mg/kg; i.p.) or saline, anaesthetised 1, 3 or 24 hours later and a high resolution anatomical scan followed by a continuous ASL (cASL) sequence was conducted using a 7 Tesla MRI scanner. Perfusion-weighted images were generated by subtraction of labelled from control images and experimental data was fitted to a quantitative model of cerebral perfusion to generate mean transit time (MTT), capillary transit time (CTT) and signal amplitude. MTT and CTT are inversely proportional to cerebral blood flow (CBF) and CBF squared respectively, and signal amplitude is proportional to cerebral blood volume (CBV). MDMA induced a reduction in MTT and CTT and an increase in signal amplitude in primary motor, secondary motor and somatosensory cortex 1 and 3 hours following administration. Such effects were not obtained in sub-cortical regions. The acute effects of MDMA on cerebral perfusion may go some way towards providing a mechanism to explain the occurrence of CVA in vulnerable recreational ecstasy users.

MDMA (20 mg/kg) provoked qualitatively similar effects to the 5-HT releasing drug fenfluramine (10 mg/kg) but not to the 5-HT_2 receptor agonist DOI (1 mg/kg). Depletion of central 5-HT produced a similar effect to that observed with MDMA-induced cortical 5-HT depletion. As 5-HT promotes vasoconstriction predominantly, a loss of the vasoconstrictive action of 5-HT might account for the increase in perfusion observed. Pre-treatment with the non selective 5-HT receptor antagonist metergoline (4 mg/kg) or with the 5-HT reuptake inhibitor citalopram (30 mg/kg), however, failed to produce any effect alone or influence the response to MDMA despite blocking MDMA-induced cortical 5-HT loss. As MDMA also provokes the release of dopamine in the brain, and dopamine may lead to vasodilatation subsequent to dopamine D_1 receptor activation on cerebral microvessels, the

effect of the dopamine D_1 receptor antagonist SCH 23390 (1 mg/kg) was also determined. While D_1 receptor antagonism provoked a decrease in cerebral perfusion in the visual and parietal association cortex, it failed to influence the changes in cortical perfusion obtained with MDMA indicating that dopamine D_1 receptors play a role in regulating blood flow in some brain regions but not MDMA-related perfusion changes in the frontal cortex. In conclusion although 5-HT depletion may play a role in mediating changes in cortical perfusion associated with MDMA administration, mechanisms independent of 5-HT such as direct drug action on, or 5-HT and dopamine D_1 receptor independent regulation of the cerebral microvasculature unit should also be considered.

Finally as repeated MDMA exposure leads to long-term 5-HT depletion, long-term changes in CBF and CBV were also assessed 8 weeks following a repeated regime of MDMA (5 and 10 mg/kg; i.p., twice daily for 4 days). Prior exposure to MDMA, having no effect alone, attenuated perfusion changes associated with acute MDMA (20 mg/kg) challenge. In addition, prior MDMA exposure was associated with a long-term reduction in cortical 5-HT concentration. The results suggest that a functional deficit develops with prior exposure in relation to cerebrovascular tone and/or neurovascular coupling in response to acute challenge. The results have implications in relation to long-term deficits in the regulation of cerebral perfusion associated with prior MDMA exposure.

In conclusion this investigation illustrates the application of btASL MRI for determination of cerebral blood perfusion changes in response to MDMA administration in a rodent model and proposes that btASL MRI is a useful investigational tool with translational potential.

III Acknowledgements

Firstly, I wish to thank my supervisor Dr. Andrew Harkin. Your constant guidance, support and insight were invaluable and I am so grateful for all of your help over the last four years.

I would also like to say a big thanks to Oli and Rustam who helped me so much with learning about the MRI and how to conduct my scans to the best of my ability. A big thanks is also due to Ann in Pharmacy who gave up so many of her mornings to help me photograph my Evans blue infused brains.

A very special thanks goes to Valentina Gigliucci for all of her dedication to making me the best "master apprentice" in HPLC that she possibly could. I never would have gotten all of it done without your help ;-).

In addition a big thank you must go to all of the members of the TC/AH lab, both past and present. In particular I would like to thank Lorna and Natacha for teaching me both lab and *in vivo* techniques that I have used over the course of my research. I would also like to say a big thanks to Áine, Jen, Katie, Eimear, Martina, Barry, Shane, Éadaoin, Raasay and Sinéad for making my years here in TCIN fun and full of good memories ☺.

Finally, I wish to express a heartfelt thank you to all of my family and friends for their support. In particular I want to thank Mum, Dad, Al and Leah for their constant encouragement, support (whether it was keeping me fed or picking me up over the course of the final few weeks) and love over the course of my time in Trinity.

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VII Abbreviations

°C	degrees Celsius
5-HIAA	5-hydroxyindole-acetic acid
5-HT	serotonin/5-hydroxytryptamine
5-HT_{1A}	type-1A serotonin receptor
5-HT_{1D}	type-1D serotonin receptor
$5\text{-HT}_{1D\alpha}$	type-1Dα serotonin receptor
$5\text{-HT}_{1D\beta}$	type-1Dβ serotonin receptor
5-HT_{2}	type-2 serotonin receptor
5-HT_{2A}	type-2A serotonin receptor
5-HT_{2B}	type-2B serotonin receptor
5-HT_{2C}	type-2C serotonin receptor
5-HT_{7}	type-7 serotonin receptor
5-HT-IR	serotonin immunoreactive
ADP	adenosine diphosphate
ANOVA	analysis of variance
ASL	arterial spin labelling
ATP	adenosine triphosphate
btASL	bolus-tracking arterial spin labelling
BBB	blood-brain barrier
BOLD	blood-oxygen level dependent
Ca^{2+}	calcium
cAMP	cyclic adenosine monophosphate

Cl^-	chloride
CBF	cerebral blood flow
CBV	cerebral blood volume
CNS	central nervous system
CTT	capillary transit time
CVA	cerebrovascular accident
D_1	type-1 dopamine receptor
D_2	type-2 dopamine receptor
D_3	type-3 dopamine receptor
D_4	type-4 dopamine receptor
D_5	type-5 dopamine receptor
DA	dopamine
DAG	diacylglycerol
DAT	dopamine transporter
DOI	1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane
EDTA	ethylenediaminetetra-acetic acid
eNOS	endothelial nitric oxide synthase
ET-1	endothelium-derived constricting factor
fMRI	functional magnetic resonance imaging
g	gram
GABA	γ-amino butyric acid
HPLC	high performance liquid chromatography
HSP90	heat shock protein
IP_3	inositol (1,4,5)-triphosphate
i.p.	intraperitoneal

IRON	increased relaxation with iron oxide nanoparticles
LCBF	local cerebral blood flow
MCA	middle cerebral artery
MDA	3,4-methylenedioxyamphetamine
MDMA	3,4-methylenedioxymethamphetamine
MRI	magnetic resonance imaging
MTT	mean transit time
mRNA	messenger RNA
NA	noradrenaline
NaH_2PO_4	sodium dihydrogen phosphate
NaCl	sodium chloride
NaOH	sodium hydroxide
ng	nanogram
NO	nitric oxide
*p*CPA	*para*-chlorophenyalanine
PKC	protein kinase C
rCBF	regional cerebral blood flow
rCBV	regional cerebral blood volume
rf	radio frequency
rpm	revolutions per minute
RT-PCR	reverse transcription-polymerase chain reaction
sec	seconds
s.c.	subcutaneous
SCH 23390	7-chloro-3-methyl-1-phenyl-1,2,4,5-tetrahydro-3-benzazepin-8-ol

SEM	standard error of the mean
SERT	serotonin transporter
SSRI	selective serotonin reuptake inhibitor
TCA	trichloroacetic acid
VMAT	vesicular monoamine transporter
v/v	volume per volume
v/w	volume per weight

Chapter 1

Introduction

Chapter 1: Introduction

1.1 3,4-methylenedioxymethamphetamine (MDMA)

1.1.1 Introduction

3,4-methylenedioxymethamphetamine (MDMA) is a readily available illicit psychoactive drug. MDMA was first synthesised and patented by the German pharmaceutical company Merck in 1914. MDMA is a synthetic drug and member of the amphetamine family of drugs. It is ring-substituted and shares a similar structure to other amphetamine derivatives including methamphetamine, 3,4-methylenedioxyamphetamine (MDA) and the hallucinogen mescaline (Figure 1.1.1).

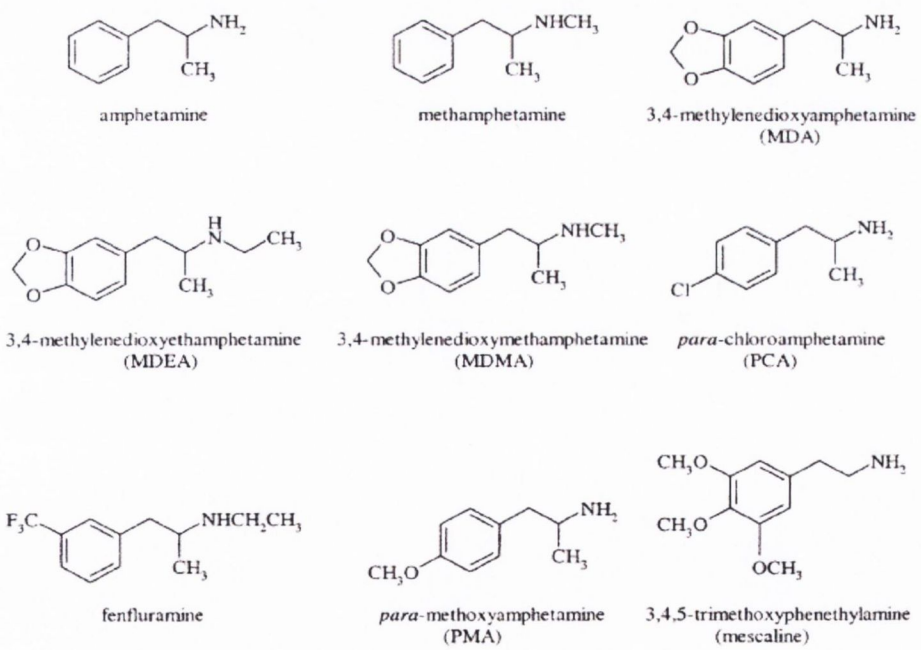


Figure 1.1.1 Structure of amphetamine and its derivatives

The substituted amphetamines differ from methamphetamine, and its parent compound amphetamine, by the presence of a methylenedioxy group attached to positions 3 and 4 of the aromatic ring of the amphetamine molecule. This group of compounds also includes 3,4-methylenedioxyethamphetamine (MDEA), and methylenedioxyamphetamine (MDA) which are closely related to MDMA and share many of its properties. Neither amphetamine nor its derivatives are found in nature and are completely synthetic substances. They structurally resemble adrenaline and dopamine, with the substituted amphetamines also resembling serotonin (5-HT), and act to enhance neurotransmitter release into the synaptic cleft. MDMA acts as a monoamine releaser, a direct and indirect monoaminergic agonist and a monoamine re-uptake inhibitor in the brain. It binds to all three of the monoamine pre-synaptic transporters (Green *et al.*, 2003), but has the highest affinity for the serotonin (5-HT) transporter (SERT) and acts mainly on the serotonergic system. It acts to a lesser extent on the dopaminergic and the noradrenergic systems resulting in increased release of these neurotransmitters. Crespi and colleagues (1997) have shown that the release of 5-HT and dopamine is both carrier-mediated and calcium-dependent (Ca^{2+}-dependent) with MDMA acting to increase cytosolic Ca^{2+} levels in neuronal terminals, thereby inducing exocytosis. MDMA also binds to various receptors and its *in vitro* pharmacological profile ranks its affinities at these receptors as follows (Battaglia *et al.*, 1988; De Souza & Battaglia, 1989): 5-HT uptake > α_2 adrenergic = 5-HT_2 = M_1 muscarinic = α_1 adrenergic = β adrenergic > dopamine uptake = 5-HT_1 >> D_2 dopaminergic > D_1 dopaminergic.

1.1.2 Recreational use of MDMA "Ecstasy"

MDMA, when used recreationally, is usually taken orally in a tablet form referred to as "ecstasy", with tablets generally containing 50 - 150 mg of the drug. The relative purity of the tablets varies and they have been shown to contain any amount of extraneous substances including caffeine, ephedrine, ketamine, paracetamol, LSD and other amphetamine derivatives (Freese *et al.,* 2002; Parrott, 2004). Patterns of ecstasy use vary between countries with a high prevalence for binge use in the United Kingdom, 25% of subjects taking 4 or more tablets per session (Winstock *et al.,* 2001).

Onset of effects are typically observed between 20 and 60 min following ingestion and peak concentrations are observed at 1.5 – 3 hr with the primary effects of the drug lasting between 3 and 5 hr (Green *et al.*, 2003). The half life ($T_{1/2}$) of MDMA is approximately 8 hr. MDMA undergoes metabolism by common metabolic pathways in the liver via several cytochrome P450 enzymes including CYP2D6 and over a dozen metabolites of MDMA have been identified in animals and humans (Green *et al.,* 2003; Kreth *et al.,* 2000). Demethylation of MDMA which produces reactive catechols is a major degradation step, as is a parallel side chain pathway initiated by N-demethylation to form MDA (Chu *et al.,* 1996). An aromatic hydroxylation pathway also exists, and has been proposed to result in the production of trihydroxymethamphetamine via 6-hydroxymethylenedioxymethamphetamine. The main metabolites of MDMA and MDA are 4-hydroxy-3-methoxymetamphetamine (HMMA) and 4-hydroxy-3-methoxyamphetamine (HMA) (Green *et al.,* 2003). An animal model of CYP2D6/D1 deficiency, the female Dark Agouti rat, is widely used in MDMA research as these rats are poor metabolisers of MDMA. Due to inefficient metabolism, brain levels of MDMA are much higher in Dark

Agouti rats when compared to Sprague Dawley rats. It has been estimated that between 5 - 10% of Caucasians are deficient in this particular enzyme and are classified as poor metabolisers (Gonzalez *et al.,* 1988). It has been proposed that poor metabolism may account for some apparently inexplicable or idiosyncratic toxic reactions to the drug (Tucker *et al.,* 1994). The kidneys are the main excretory organs and MDMA has a non-linear pharmacokinetic profile, probably due to either a saturable or inhibitable metabolic pathway (de la Torre *et al.,* 2004; Farré *et al.,* 2004) increasing the chance of accidental overdose. There are 2 stereoisomers of MDMA with S(+) being metabolised faster than R(-) and demonstrating greater neurotoxicity in the rat (Kalant, 2001).

Increased synaptic 5-HT availability is believed to be responsible for the feelings of euphoria and enhanced confidence in addition to increased feelings of serenity and calmness (Liechti *et al.,* 2000a,b; Verheyden *et al.,* 2003). Many studies have reported a rapid increase in release of 5-HT following MDMA administration using *in vivo* microdialysis (Mechan *et al.*, 2002; Shankaran and Gudelsky, 1999) and using *in vitro* approaches (Koch and Galloway, 1997; O' Loinsigh *et al.*, 2001). This is followed by a pronounced decrease in brain levels of 5-HT and its primary metabolite, 5-hydroxyindoleacetic acid (5-HIAA) and the activity of the 5-HT synthesising enzyme tryptophan hydroxylase. Within 24 hr brain 5-HT levels recover to normal baseline values but 3 days following drug administration a sustained and regionally specific depletion of 5-HT and 5-HIAA is seen which has been shown to persist for up to 12 months in the rat (Battaglia *et al.,* 1987; Baumann *et al.,* 2007; Harkin *et al.,* 2001; McKenna and Peroutka, 1990; O'Shea *et al.,* 1998; Ricaurte *et al.,* 2000; Shankaran & Gudelsky, 1998).

With respect to dopamine (DA) release, there is evidence that MDMA elicits this effect via 5-HT release (Koch and Galloway, 1997) via the 5-HT_{2A} receptor (Nash, 1990) and via a carrier-mediated mechanism independent of 5-HT release (Nash and Brodkin, 1991). The positive effects of MDMA decrease while the negative effects increase with respondents reporting an increasing tolerance to the drug with repeated use (Solowij *et al.,* 1992; Winstock *et al.,* 2001).

1.1.3 MDMA toxicity

The toxicity of MDMA which is exhibited both peripherally and centrally has been extensively reviewed elsewhere (Green *et al.,* 2003). It has been estimated that ingestion of the drug results in the deaths of 15 persons per year in the UK. Nevertheless at the height of its popularity, when approximately 500,000 people consumed the drug in an uncontrolled manner every week in the UK, it became evident that MDMA is actually not very dangerous or toxic in the short-term. The major concern relating to MDMA is its putative long-term neurotoxic effects that may not be apparent for many years after consumption.

In combination with the psychological effects associated with the ingestion of MDMA, many physiological changes have also been reported including a change in core body temperature. Hyperthermia is one of the defining features of ecstasy use in humans and body temperatures in excess of 43°C have been reported following emergency room admissions associated with the drug (Henry, 1992; Henry *et al.,* 1992). Similarly a major effect of MDMA administration to experimental animals is a rapid hyperthermia that is most robust 40 - 60 min after administration and which can persist for several hours (Green *et al.,* 2003). Under normal ambient temperatures of 20 - 22°C MDMA administration has

been reported to cause a marked hyperthermic response (Che *et al.*, 1995; Dafters, 1994; O'Shea *et al.*, 1998). However, at ambient temperatures below 20 - 22°C, a hypothermic response has been observed following MDMA administration to rats (Marston *et al.*, 1999). Dafters & Lynch (1998) observed that ambient temperatures of 17°C resulted in a hypothermic response in rats following administration of MDMA (10 - 15 mg/kg; s.c.). These findings indicate that MDMA has a profound effect on thermoregulatory mechanisms and that the substance is highly sensitive to external temperature changes. It has been reported that acute 5-HT release is not directly responsible for hyperthermia, but that 5-HT receptors modulate the response (Docherty & Green, 2010; Green *et al.*, 2004, for review). In addition dopaminergic D_1 receptors and α_1-, α_{2A}- and β_3- adrenoceptors have been implicated in the MDMA-induced hyperthermic response (Docherty & Green, 2010). It is thought that MDMA may compromise thermoregulation or the body's ability to maintain a stable core body temperature despite changes in ambient temperature. This is of considerable relevance to human MDMA use as the vast majority of MDMA consumption occurs at "raves" where a high ambient temperature, overcrowding and excessive dancing greatly influence the effects of the drug (Parrott, 2011).

In addition to the thermoregulatory changes observed following MDMA administration further physiological changes have been reported. In rats "serotonin syndrome" is observed following administration of MDMA. This behavioural syndrome includes hyperlocomotion, flattened body posture, head weaving, piloerection, hind limb retraction, straub tail, sweating and forepaw treading (Colado *et al.*, 1993; de Souza *et al.*, 1997; Marston *et al.*, 1999; Shankaran & Gudelsky, 1999). MDMA administration to animals affects a variety of other behaviours including those related to anxiety and cognition (Cole & Sumnall 2003a,b; Green *et al.*, 2003, for reviews). MDMA was reported to have cardiac

stimulatory effects resulting in tachycardia and arrhythmia in rats (Dumont *et al.,* 2009; Gordon *et al.,* 1991; O'Cain *et al.,* 2000; Vanattou-Saifoudine *et al.*, 2010b) and increased blood pressure (Broadley, 2010). The number of MDMA associated hospital admissions presenting with cardiovascular toxicity suggests that MDMA profoundly affects parameters such as heart rate and arterial pressure (Henry *et al.,* 1992) however, the cardiovascular actions of MDMA have not been well characterised. It is difficult to carry out studies in humans mimicking the uncontrolled conditions the drug is normally taken under, such as overcrowding, excessive dancing and loud music. From clinical studies that have been conducted – albeit in a more controlled environment than that in which the drug is typically consumed – MDMA has been shown to produce a modest tachycardia and hypertension (Downing, 1986; Mas *et al.,* 1999; Verheyden *et al.*, 2003; Vollenweider *et al.,* 1998) although these studies also reported severe responses in certain individuals. When administered acutely in recreational doses to human volunteers (0.25 – 1.9 mg/kg; p.o.), MDMA increased cardiovascular activity, which peaked between 1 and 2 hr following administration (de la Torre *et al.,* 2000a,b; Lester *et al.,* 2000; Liechti & Vollenweider, 2000a,b). It could therefore be the case that MDMA exacerbates latent cardiovascular problems and could pose serious threats in a dance club setting. The physiological changes alluded to here are the most common indicators of MDMA-induced toxicity.

1.1.4 MDMA neurotoxicity

With respect to long-term effects, 5-HT neurons appear to be almost exclusively susceptible to damage by MDMA in primates and rats (Bankson & Cunningham, 2001; Colado *et al.,* 2004; Green *et al.,* 2003; Gudelsky & Yamamoto, 2008; Shankaran & Gudelsky, 1998). SERT density, a hallmark of 5-HT nerve terminal integrity, is reduced

following MDMA administration. Battaglia and colleagues (1987) and others (McCann *et al.,* 1998; Reneman *et al.,* 2001) have reported significant reductions (up to 60 - 70%) in 5-HT uptake sites following MDMA (20 mg/kg; s.c., twice daily for 4 consecutive days) administration to rats in comparison to vehicle treated control animals, indicative of a reduction in 5-HT nerve terminal integrity. Immunoreactive 5-HT axon density was quantified by Hatzidimitriou and colleagues (1999) in various brain regions following MDMA administration (5 mg/kg; s.c., twice daily for 4 consecutive days) to non-human primates. 83 - 95% reductions in 5-HT immunoreactive (5-HT-IR) axon density were reported in cerebral cortex two weeks following MDMA administration. Seven years after treatment with MDMA, reductions in 5-HT-IR were still evident but significant recovery had occurred in comparison to the two week response. There are two major 5-HT projections from the raphe nuclei to forebrain areas and immunocytochemistry studies in animals have shown a differential vulnerability to the neurotoxic effects of MDMA. Fine 5-HT axons arising from the dorsal raphe nucleus display an enhanced vulnerability while beaded 5-HT axons originating from the median raphe nucleus are spared (Mamounas & Molliver, 1988; Molliver *et al.,* 1990). Retrograde degeneration does not seem to occur, leaving cell bodies in the raphe nuclei intact and there is evidence that damaged terminals can recover (Battaglia *et al.,* 1988; Mayerhofer *et al.,* 2001). MDMA administration to mice also results in changes, to a lesser extent, in the concentration of the catecholamines, dopamine (DA) (Bankson & Cunningham, 2001; Colado *et al.,* 2004; Green *et al.,* 2003; Gudelsky & Yamamoto, 2008; Shankaran & Gudelsky, 1998) and noradrenaline (NA) (Green *et al.,* 2003; Rothman *et al.,* 2001).

There is also accumulating evidence in support of 5-HT neurotoxicity associated with MDMA use in humans. A post mortem study carried out by Kish and colleagues (2000)

showed a 50 - 80% depletion of 5-HT and 5-HIAA in the brain of a chronic MDMA user, while dopamine concentrations were unaffected. In addition, human studies using neuroimaging techniques have indicated 5-HT neuronal damage following MDMA administration (McCann *et al.,* 1998; Obrocki *et al.,* 2002; Semple *et al.,* 1999) but caution is advised in relation to the interpretation of these findings (de Win *et al.,* 2004; Kish, 2002; Thomasius *et al.,* 2003). Impairment of 5-HT function is also supported by blunted responses to challenge with the 5-HT releasing agent D-fenfluramine (Gerra *et al.,* 1998; 2000) and reduced 5-HIAA in the cerebrospinal fluid of abstinent MDMA users (McCann *et al.,* 1994). There has been some speculation that MDMA itself does not mediate the neurotoxicity (Esteban *et al.,* 2001; Paris & Cunningham, 1992) and that it may, in fact, be the products of metabolism which are taken into the 5-HT neuron which are responsible (Bai *et al.,* 2001; Cadet & Brannock, 1998; Capela *et al.,* 2007; Carvalho *et al.,* 2004; Colado *et al.,* 1995; de la Torre *et al.,* 2004; Jones *et al.,* 2005). In addition oxidative stress has also been implicated in MDMA-induced neurotxicity (Puerta *et al.,* 2010; Steinkellner *et al.,* 2011; Yamamoto & Raudensky, 2008).

1.2 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is an imaging technique which may be employed in both clinical and pre-clinical investigations to obtain a high quality image of the interior of the brain.

The use of MRI, in humans and animals, is possible due to the fact that body tissues are comprised of a high proportion of both water and fat. Both of these substances contain

large numbers of hydrogen atoms which comprise unpaired protons. These unpaired protons possess a phenomenon known as "spin". The spin of an unpaired proton allows protons to line up with (parallel formation) or against (anti-parallel formation) a magnetic field, following application of a magnetic field. In MRI, a magnetic field is generated by a magnetic field gradient coil (Figure 1.2).

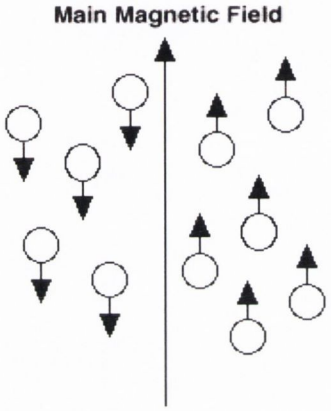


Figure 1.2 Protons, or hydrogen ions, aligning parallel and anti-parallel to a magnetic field

These protons possess different energy states and a proton has the ability to move from one energy state to another energy state following the absorption of a photon. When the energy of the photon matches the energy difference between the two spin states, absorption of energy occurs. In MRI, the frequency of the photon falls within the radio frequency (RF) range (www.cis.rit.edu/htbooks/mri/inside.htm) and this frequency may be applied by a radiofrequency coil. When the RF pulse is turned off, the hydrogen protons return to their natural alignment within the magnetic field and release their excess stored energy. When this occurs a signal is released from the protons which the coil receives. The signal is

subsequently integrated and converted through the use of a Fourier transformation into an MR image (Huettel *et al.*, 2008).

1.3 Arterial Spin Labelling

Arterial spin labelling (ASL) is a method used to assess for functionality within an MRI scan. It acts to assess cerebral blood flow (CBF) or cerebral blood volume (CBV) in the brain without the use of neuronal activation. It is a technique originally introduced by Alsop & Detre (1996) and it is the only MRI technique that can directly and absolutely quantify regional CBF (rCBF) (Beckmann, 2006). An MR image can become sensitive to CBF changes if the magnetic state of blood water spins is different to that of the tissue water spins (Thomas *et al.*, 2000). This ASL technique uses magnetically labelled arterial blood water as an endogenous tracer for the assessment of perfusion changes (Jahng *et al.*, 2007). In this way ASL MRI is a non-invasive imaging technique that assesses for cerebral blood perfusion changes. This method is advantageous as it causes minimal disturbance to the system being imaged (Beckmann, 2006). Two separate sets of MR images are generated following an ASL scan. The first image contains blood and tissue water magnetisations that are different (the *labelled* image) and the second image contains blood and water magnetisations that are the same (the *control* image). Subtraction of the *labelled* from the *control* image generates a perfusion weighted image with an intensity that is directly related to perfusion (Thomas *et al.*, 2000).

Recently, a new quantitative bolus-tracking ASL (btASL) MRI technique was developed and described by Kelly and colleagues (2009) for the measurement of perfusion state in the

rodent brain. The technique assesses cerebral perfusion through the calculation of two transit times: the mean transit time (MTT) which represents the time taken for labelled arterial blood water to traverse the vasculature and reach the imaging plane and the capillary transit time (CTT) which represents the time taken for the arterial blood water to disperse at the imaging plane. MTT is inversely proportional to CBF, while CTT is inversely proportional to CBF squared. A third quantifiable output is the btASL signal amplitude, which is derived from the area under the signal-time ASL curve and has been interpreted as being proportional to CBV (Figure 1.3).

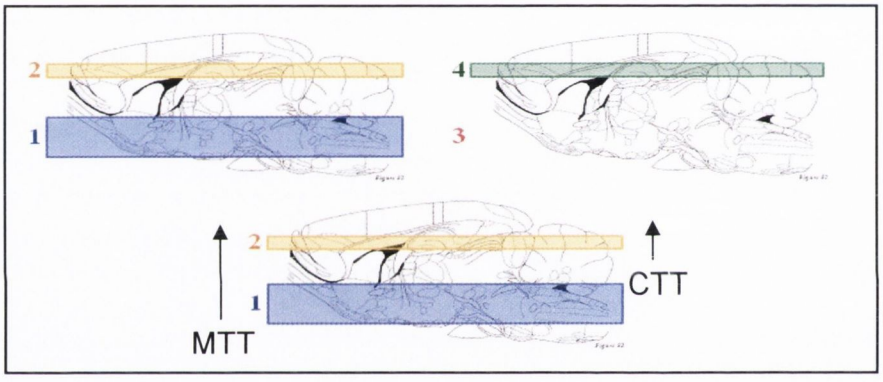


Figure 1.3 Schematic depicting the btASL MRI technique

The schematic depicts ASL as it occurs. Briefly:

1. The inflowing arterial blood water is magnetised.

2. An image of this magnetised blood is taken at the imaging plane (the *labelled* image).

3. The inflowing arterial blood water has no magnetic pulse applied to it.

4. An image of this unlabelled blood is taken at the imaging plane (the *control* image). Subtraction of the *labelled* from the *control* image generates a perfusion weighted image, used for the assessment of CBF and CBV changes.

1.4 Cerebral Blood Flow

1.4.1 Introduction

CBF is the blood supply to the brain at any given time. The brain is dependent on a continuous supply of oxygenated blood and it has the ability to control the blood delivery by sensing pressure changes in its main arteries and by monitoring respiratory gas levels. The major arteries supplying the brain are the internal carotid arteries which divide into the anterior and middle cerebral arteries. The basilar artery divides into the two posterior cerebral arteries at the upper border of the pons and the Circle of Willis links all of these arteries. The arteries which arise from this structure branch out into smaller pial vessels that bring blood to the brain surface. The pial arteries (Figure 1.4.1) give rise to penetrating arteries and arterioles which penetrate the substance of the brain and as the arterioles become progressively smaller with each branching, by losing their smooth muscle layer, they become cerebral capillaries. These capillaries are also known as the intracerebral or intraparenchymal micro vessels (Cohen *et al.*, 1996) and all the other vessels in the brain including the pial vessels and the major cerebral arteries are known as the extracerebral vessels (Cohen *et al.*, 1996). The endothelial cells of these capillaries are not fenestrated, as they are in the periphery, but are instead inter-connected by focal adhesions (Iadecola *et al.*, 2004) known as tight junctions which, along with the astrocytic end-feet, form the blood brain barrier (BBB). The BBB is extremely important in the brain as it modulates the entry of metabolic substances such as glucose, controls the movement of ions, and prevents the access of toxins and peripheral neurotransmitters to the central nervous system. The presence of the BBB is one of the major differences that exists between the peripheral and the cerebral vasculature.

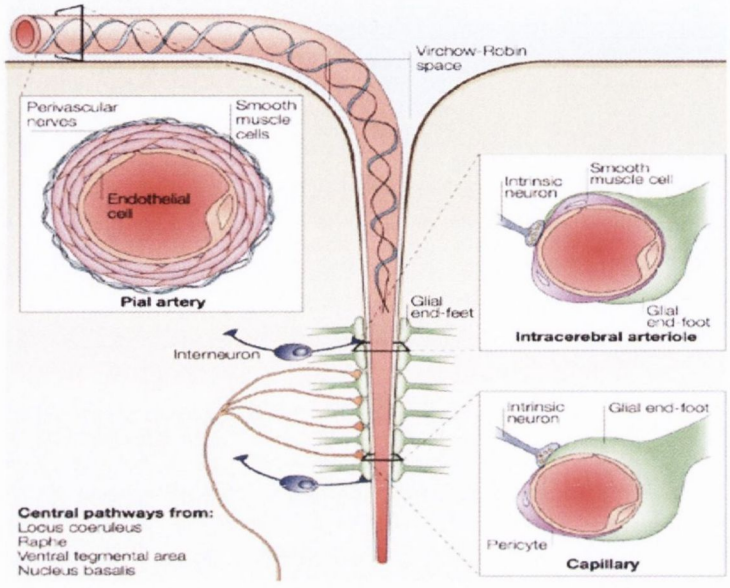


Figure 1.4.1 Schematic of brain vasculature (Iadecola et al., 2004)

The diagram shows a pial artery branching into the intracerebral arteriole and finally the capillary. In addition, it shows the connections of the vessels to cells.

1.4.2 Neurovascular Coupling

In the brain energy must be made available where needed quickly and efficiently to ensure proper functioning and this means blood flow must correlate closely with neuronal activation. A useful conceptual tool in describing this phenomenon is the neurovascular unit which is the functional unit comprising neurons, blood vessels, and glial cells that work in unison to ensure adequate blood flow is coupled to neuronal activation (Drake & Iadecola, 2007). Neurovascular coupling enables cerebral blood flow to be increased in areas of the brain that are active and it has been associated with many of the classical

neurotransmitters such as acetylcholine, glutamate, GABA, 5-HT, dopamine and noradrenaline as well as some non-conventional transmitters such as nitric oxide (NO).

Our understanding of the processes involved in the regulation of CBF is evolving and subject to debate (Paulson *et al.,* 2010). It is not always clear exactly how activation and blood flow are coupled and this may be due to limitations in the ability to measure neuronal activity or CBF and to correlate them accurately (Tan, 2009). The current paradigm is that rather than being controlled by a negative feedback loop (an energy deficit signalling for increased blood flow), feed-forward mechanisms (increased neuronal firing amplifying local and upstream blood flow) are key in second-to-second changes in CBF (Cauli & Hamel, 2010). However, it is becoming clear that astrocytes (Haydon & Carmignoto, 2006), pericytes (Kamouchi *et al.,* 2011), local neurons (Drake & Iadecola, 2007), and the direct and indirect effects of neurotransmitters (Cauli & Hamel, 2010) play a key role in the process.

1.4.3 Regulation of Cerebral Blood Flow

(a) Endothelial Cells

The endothelial cells of the microvessels are regulators of vascular tone, vasculogenesis, inflammation and thrombosis (Andresen *et al.*, 2006). Stimulation of endothelial cells leads to the production of vasoactive factors. Acetylcholine, bradykinin, statins, oestrogen, adenosine diphosphate (ADP) and adenosine triphosphate (ATP) all activate G-protein coupled receptors located on the endothelium which can stimulate vasodilatation by production of NO. Ca^{2+} concentration and heat shock protein (HSP-90) (Andresen *et al.*, 2006) are

regulators of endothelial nitric oxide synthase (eNOS) and so a decrease in the Ca^{2+} concentration or inhibition of the HSP-90 protein can lead to constriction of cerebral blood vessels. Three endothelium-derived constricting factor (ET-1) receptors have been found expressed in the endothelium and smooth muscle (Andresen *et al.*, 2006). ET-1 is a profound vasoconstrictor that increases intracellular Ca^{2+} concentration and may also increase release of chloride (Cl^-) from smooth muscle cells (Andresen *et al.*, 2006).

Endothelial-derived vasoactive compounds are of importance because neurotransmitters such as acetylcholine (Heistad *et al.*, 1977), dopamine (Krimer *et al.*, 1998), 5-HT (Cohen *et al.*, 1996) and noradrenaline (Raichle *et al.*, 1975) have all been shown to have either vasoconstrictor or vasodilatatory properties and endothelial vasoactive compounds may play a role in their mechanisms of action.

(b) Pericytes

Recently a role for pericytes has been identified in the control of blood vessel diameter. Pericytes, or contractile cells, have been found on almost all capillaries, arterioles and venules and were initially thought to be an important constituent of support and scaffolding of the blood vessel (Dore-Duffy, 2008). However, it now seems that pericytes have a number of roles to play including the formation of new blood vessels, the stabilisation of blood vessels, endothelial cell regulation and maintenance of BBB (Kamouchi *et al.*, 2004; Hamilton *et al.*, 2010). Pericytes are thought to be mulitpotent precursors and it has been suggested that they are the precursors for several different cells including neuroglia (Dore-Duffy, 2008). Studies of pericytes from other adult organs such as the skin and the liver have shown that these cells do indeed form progenitor skin and liver cells (Dore-Duffy, 2008; Hamilton *et al.*, 2010).

In addition, pericytes appear to have a macrophage-like activity acting as first line defence in the brain and having the ability to present antigen (Guillemin & Brew, 2004). The pericytes have processes that surround the capillaries (Peppiatt *et al.*, 2006) and the primary processes extend from the pericyte cell body along the capillary and subsequently branch into secondary and tertiary processes (Hamilton *et al.*, 2010). Their importance within the nervous system is further supported by the fact that there are more pericytes per endothelial cell here than in any other area of the body (Hamilton *et al.*, 2010).

The main evidence that supports pericytes as contractile cells derives from *in vitro* experimentation. Kamouchi and colleagues (2004) isolated brain capillaries, with no smooth muscle, from five week old male rats. The cells were allowed to grow and, within days, colonies of pericytes proliferated from each capillary. The pericytes were sub-cultured, and addition of 5-HT significantly reduced the surface area of these cells. In addition, it was found that 5-HT caused a biphasic increase in intracellular Ca^{2+} concentration when applied to the pericytes. These findings provide evidence to indicate a role for pericytes as contractile cells. Further evidence for this comes from the expression of the smooth muscle-specific isoform of actin, α smooth muscle actin (αSMA) (Dore-Duffy, 2008; Hamilton *et al*, 2010). Contraction by pericytes is thought to use similar mechanisms to those found in smooth muscles including contraction being evoked by a rise in Ca^{2+} concentration which results in Ca^{2+}-calmodulin dependent activation of myosin light chain kinase which phosphorylates the myosin light chain and promotes interaction with αSMA. Relaxation is due to low Ca^{2+} concentration which has the opposite effect in the cell.

Astrocytic end-feet and neurons have both been shown to interact with pericytes suggesting they may also have a role within the neurovascular unit (Kamouchi *et al.*, 2004; Bergers & Song, 2005; Dore-Duffy, 2008; Hamilton *et al.*, 2010). Pericyte-induced vasoconstriction may also be caused by an increase in intracellular Ca^{2+} concentration leading to an increase in resting membrane potential which subsequently activates L-type voltage-operated Ca^{2+} channels (VOCCs). In addition, activation of non-specific cation channels, leading to the activation of VOCC, and Ca^{2+}-activated Cl^- channels can increase the activity of the voltage-operated channels (Hamilton *et al.*, 2010), and it is through these aforementioned mechanisms that pericytes can act to mediate CBF.

(c) Neurotransmitters

The neurotransmitter 5-hydroxytryptamine (serotonin; 5-HT) has been implicated in the regulation of CBF. 5-HT is a well characterised neurotransmitter in the CNS. It is synthesised from the amino acid tryptophan which is taken up into the nerve terminal and converted to 5-HT which is subsequently stored in vesicles by the vesicular monoamine transporters (VMATs). Release of 5-HT from the nerve terminal leads to the activation of 5-HT receptors. To date 16 different 5-HT receptors have been identified (Rho and Storey, 2001) which have been divided into seven distinct classes 5-HT_1 to 5-HT_7. All receptors, with the exception of the 5-HT_3 receptor, are G-protein coupled receptors. 5-HT_1 receptors are negatively coupled to adenylate cyclase while the 5-HT_4, 5-HT_6 and 5-HT_7 receptors stimulate production of adenylate cyclase. The 5-HT_2 receptors are coupled to phosphatidylinositol. 5-HT is removed from the synaptic cleft via the pre-synaptically located SERT. 5-HT neurons originate primarily from the raphe region of the upper pons and brainstem and have widespread forebrain projections.

5-HT neurons have been reported to modulate blood flow in the microcirculation (Cohen *et al.*, 1996). Ultrastructural analysis has revealed an intimate association between serotonergic neurons and blood vessels in the brain (Cohen *et al.*, 1995; Milner *et al.*, 1966). It was the discovery of this association that promoted the theory that 5-HT could modulate cerebral blood flow. The vasoconstrictive actions of 5-HT have been widely reported in both humans (Kaumann *et al.*, 1993; Price *et al.*, 1997; Ullmer *et al.*, 1995) and animals (Cao *et al.*, 1992; McBean *et al.*, 1991; Roberts *et al.*, 1997) however, 5-HT has also been reported to promote vasodilatation under certain conditions (Cohen *et al*, 1996). The predominant vasomotor effect of 5-HT on cerebral blood vessels is constriction and it has been suggested that the different receptor subtypes, and possibly the vessel tone before exposure, mediate the opposing effects of 5-HT on the microcirculation.

A high number of perivascular serotonergic neurons synapse on astrocytes, implying an important role for astrocytes in the regulation of CBF. Astrocytes have been shown to express a number of 5-HT receptor subtypes including 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} (Cohen *et al.*, 1996; Osredkar & Krzan, 2009). The 5-HT_{1A} receptors act by inhibiting adenylate cyclase which decreases cyclic adenosine monophosphate (cAMP) leading to down-regulation of particular genes. This can lead to reduced levels of cyclooxygenase eicosanoids (Volterra & Meldolesi, 2005) which can result in vessel constriction. The 5-HT_{2A} receptors are associated exclusively with astrocytes in the human brain (Cohen *et al.*, 1995). These along with the 5-HT_{2C} receptors increase levels of inositol trisphosphate (IP_3) and diacylglycerol (DAG) which can activate membrane bound protein kinase C (PKC) (Golan *et al.*, 2008). The rise in IP_3 causes release of intracellular Ca^{2+}, which produces vasoconstriction mediated by second messengers (Girouard & Iadecola, 2006; Golan *et al.*,

2008). 5-HT has also been shown to produce constriction via pericytes on smaller cerebral blood vessels, and smooth muscle cells on the larger cerebral blood vessels. These cell types have been identified to express 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, 5-HT_{2B} and 5-HT_7 receptor types (Cohen *et al.*, 1995). All of these cells induce contraction of the vessel when their receptors are activated by 5-HT via Ca^{2+} influx pathways (Hamilton *et al.*, 2010; Kamouchi *et al.*, 2004; Peppiatt *et al.*, 2006). The 5-HT receptors on pericytes are likely to induce constriction of cerebral blood vessels in the same manner described previously. Thus, 5-HT is a potent modulator of CBF in the microcirculation and is capable of constricting vessels using a variety of mechanisms and through a number of different cell types.

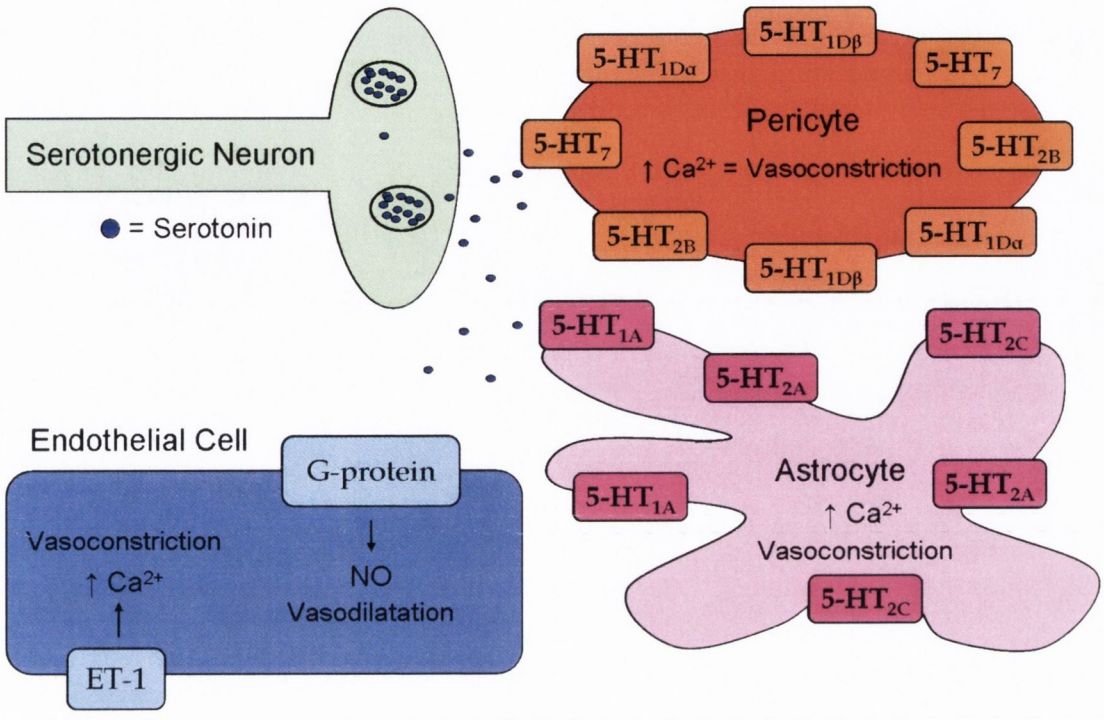


Figure 1.4.3 Schematic of the neurovascular unit and the role it has to play in modulating cerebral blood flow

The serotonergic neuron, following stimulation, releases 5-HT which modulates vasoconstriction. The presence of 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, 5-HT_{2B} and 5-HT_7 receptors on pericytes mediate vasoconstriction through an increase in intracellular Ca^{2+} concentration. The 5-HT_{1A} receptors on astrocytes act by inhibiting adenylate cyclase which decreases cyclic adenosine monophosphate (cAMP) leading to down-regulation of particular genes. This can lead to reduced levels of cyclooxygenase eicosanoids which can result in vessel constriction. The 5-HT_{2A} and 5-HT_{2C} receptors increase levels of inositol trisphosphate (IP_3) and diacylglycerol (DAG) and this rise in IP_3 causes release of intracellular Ca^{2+}, which produces vasoconstriction mediated by second messengers. Stimulation of the endothelial cells produces vasoactive factors including acetylcholine, bradykinin, statins,

oestrogen, adenosine diphosphate (ADP) and adenosine triphosphate (ATP) which activate G-protein coupled receptors which can stimulate vasodilatation by the production of nitric oxide (NO). Endothelium-derived constricting factor (ET-1) receptors have been found expressed on the endothelium and ET-1 is a profound vasoconstrictor that increases intracellular Ca^{2+} concentration.

The catecholamine dopamine has been implicated in the regulation of CBF. Dopamine is a catecholamine and acts as a neurotransmitter in the CNS. It is synthesised from the amino acid tyrosine which is taken up into the nerve terminal and converted to dopamine which is subsequently stored in vesicles by the vesicular monoamine transporters (VMATs). Release of dopamine from the nerve terminal leads to the activation of dopamine receptors. To date 5 different dopamine receptors have been identified (Golan *et al.*, 2008) which have been divided into 2 distinct classes D_1-like and D_2-like. D_1-like comprise D_1 and D_5 receptors whereas D_2-like comprise D_2, D_3 and D_4 receptors. D_1-like receptors are positively coupled to G-proteins leading to stimulation of adenylate cyclase and D_2-like receptors are negatively coupled to G-proteins leading to inhibition of adenylate cyclase. Dopamine is removed from the synaptic cleft via the pre-synaptically located dopamine transporter (DAT).

Dopamine is involved in a wide and diverse variety of physiological, psychological, and behavioural processes including movement, reward, addiction (Volkow *et al.,* 2009a,b), learning, perception, and creativity (Egerton *et al.,* 2009). There are five dopamine pathways in the brain which diverge extensively to a great number of brain regions. The first is the mesolimbic-mesocortical pathway which projects from cell bodies near the

substantia nigra to the limbic system and the neocortex and is implicated in behaviour. The second is the nigrostriatal pathway which consists of neurons projecting from the substantia nigra to the caudate/putamen and is involved in voluntary movement. The third is the tuberoinfundibular pathway, which links arcuate nuclei and periventricular neurons to the hypothalamus and posterior pituitary and is involved in prolactin homeostasis. The fourth is the medullary-periventricular pathway which consists of cell projections in the motor nucleus of the vagus nerve. The fifth is the incertohypothalamic pathway which connects the medial zona incerta to the hypothalamus and the amygdala (Golan *et al.*, 2008).

Krimer and colleagues (1998) first speculated that observed changes in vessels innervated by dopaminergic neurons were due to direct effects of dopamine on the vessel rather than due to neuronal activation. Further studies have been carried out to elucidate a role for dopamine in mediating CBF changes. Utilising an array of selective dopamine agonists, antagonists, releasers and re-uptake inhibitors, and using both increased relaxation with iron oxide nanoparticles (IRON) and blood-oxygen level dependent (BOLD) techniques investigators examined the effects of dopamine release on the cerebrovasculature to elucidate the specific DA receptors underlying the observed changes. Investigators reported a strong correlation between dopamine concentration in the brain, as released by amphetamine, and relative cerebral blood flow (rCBF). Administration of a dopamine transporter (DAT) blocker produced comparable results (Krimer *et al.*, 1998). Amphetamine administration alone, and in combination, with a D_1/D_5 antagonist (SCH 23390) revealed that SCH 23390 produced a small negative rCBF change (approximately 5%) when administered alone, but blocked the rCBF changes normally induced by amphetamine and DAT blocker. D_2 agonists (quinpirole and R(-)-2,10,11-trihydroxy-N-propylnorapomorphine hydrobromide) were reported to induce negative rCBF changes in

the regions where D_2 receptors are present. The D_3 agonist 7-OHDPAT also produces small negative rCBF changes, but differed from D_2 agonists in respect that no CBF changes were observed in the caudate/putamen. The findings of this study indicate that increases in CBF are mediated by agonism of D_1/D_5 receptors, while decreases in rCBF are mediated by agonism of D_2/D_3 receptors (Choi *et al.,* 2006). Ren and colleagues (2009) also reported a strong relationship between amphetamine dose and dopamine release however, a negative rCBF change was associated with low dose amphetamine. Microdialysis, performed to assess dopamine release at low amphetamine concentration, indicates a dose-dependent release of dopamine. It was hypothesised that, because D_2/D_3 receptors have a higher affinity for dopamine than D_1/D_5 receptors, at low concentrations, they have higher relative occupancy and thus exert more net effect on the vasculature. As amphetamine, and thus dopamine, concentration increases, D_1/D_5 receptors dominate the vascular effect. These studies provide evidence to imply a role for dopamine as a potent modulator of CBF in the microcirculation.

The catecholamine noradrenaline has been implicated in the regulation of CBF. Bryan and colleagues (1996) reported a role for α_2-adrenoceptors in mediating vasodilatation in rat middle cerebral artery (MCA). Arteries were harvested and vessel diameter was measured following a variety of pharmacological challenges. Authors reported a role for α_2-adrenoceptors due to the fact that UK 14,304, an α_2-adrenoceptor agonist, produced dose-dependent increases in vessel diameter. In addition to this, the α_2-adrenoceptor antagonists idazoxan and rauwolscine attenuated and blocked, respectively, UK 14,304-induced MCA vasodilatation. The presence of α_1-adrenergic receptor subtypes have also been identified in rat cerebral microvessels (Yokoo *et al.,* 2000). Reverse transcription-polymerase chain reaction (RT-PCR) experiments revealed that messenger RNA (mRNA) for α_{1A}- and α_{1B}-, but

not α_{1D}- adrenoceptors, was expressed in the cerebral microvessels. [^{125}I] iodo-2-[β-(4-hydroxyphenyl)-ethyl-aminomethyl]tetralone ([^{125}I]HEAT) binding to the cerebral microvessels was inhibited by 5-methylurapidil, a selective α_{1A}-adrenergic receptor antagonist, in a dose-dependent manner.

1.5 Summary

It has been reported that MDMA "ecstasy" abuse may lead to CVA (Harries & De Silva, 1992; McEvoy *et al.,* 2000; Perez *et al.,* 1999; Petitti *et al.,* 1998). For instance, intracerebral haemorrhage in the left frontoparietal region has been reported in a 21 year old female (Hughes *et al.,* 1993). In addition, right-sided subarachnoid haemorrhage has been reported in an 18 year old following use of ecstasy (Auer *et al.,* 2002). Although CVA have been reported to be associated with MDMA use, the exact mechanism by which this occurs has not been fully elucidated. It has been suggested that such events may be related to MDMA-induced changes to the cerebrovasculature and cerebrovascular function and that these effects are likely to be mediated by changes to chemical events such as neurotransmitter release, which have a role to play in modulation of the microvasculature and neurovascular unit (Chang *et al.,* 2000; Ferrington *et al.,* 2006; van Donkelaar *et al.,* 2010). Investigations into the long-term effects of MDMA on CBF and CBV are of utmost importance to enhance our understanding of the long-term perfusion alterations associated with chronic MDMA abuse.

1.6 Aims and Objectives

The overall aim of the work described in this thesis was to determine the acute and long-term effects of MDMA on cerebral blood perfusion and to explore the underlying mechanisms in a rodent model of MDMA abuse using btASL MRI. Specifically the objectives were as follows:

(1) To employ btASL MRI to determine regional, time and dose-dependent changes to cerebral perfusion in the rat following acute MDMA administration. It was also deemed necessary to clarify if any changes observed were associated with BBB disruption as sustained increases in BBB permeability have previously been reported following administration of high doses of MDMA to rats.

(2) Given the established role of 5-HT and dopamine in the regulation of cerebral perfusion, attempts were made to determine the mechanisms that mediate the ability of MDMA to increase cortical perfusion and volume in rats. First, the ability of MDMA to generalise to fenfluramine, a synthetic amphetamine that selectively induces the release of central 5-HT was determined, or if the response to MDMA could be simulated by administration of the non-selective 5-HT_2 receptor agonist 2,5 dimethoxy-4-iodophenyl-aminopropane hydrochloride (DOI). Next, the effects of 5-HT depletion on MDMA-induced changes in cortical perfusion were determined. In addition, inhibition of 5-HT transmission was assessed by prior administration of the non-selective 5-HT receptor antagonist metergoline. The consequences of blocking the 5-HT transporter and resultant uptake of MDMA with citalopram for cortical perfusion changes associated with MDMA were assessed. Finally to elucidate a role for dopamine D_1 receptors, the effect of prior

administration of the selective dopamine D_1 receptor antagonist SCH 23390 was assessed on MDMA-induced changes.

(3) To determine if repeated exposure to MDMA with subsequent long-term central 5-HT loss may provoke sustained alterations in cerebral cortical perfusion and blood volume in the rodent model established. Moreover it was of interest to determine if prior exposure to MDMA and subsequent long-term cortical 5-HT loss would influence the response to acute challenge with MDMA.

Chapter 2

Materials and Methods

2.1 Materials

2.1.1 Animals

Wistar rats	Bioresources, Trinity College Dublin

2.1.2 Experimental treatments

Citalopram	Gerard laboratories, Ireland
D-fenfluramine	Sigma Aldrich, Ireland
DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane)	Sigma Aldrich, USA
MDMA (3,4-methylenedioxymethamphetamine)	NIDA, USA
Metergoline	Sigma Aldrich, Ireland
*p*CPA (*para*-chlorophenylalanine)	Sigma Aldrich, Ireland
SCH 23390	Sigma Aldrich, Ireland

2.1.3 High Performance Liquid Chromatography reagents

5-hydroxyindole-3-acetic acid (5-HIAA)	Sigma Aldrich, Ireland
Citric acid ($C_6H_8O_7$)	BDH Chemicals, Poole, U.K.
Ethylenediaminetetra-acetic acid (EDTA)	BDH Chemicals, Poole, U.K.
HPLC grade water	Fisher Chemical, U.K.
Methanol, 100%	Lab-Scan, Ireland
N-methy-5-HT	Sigma Chemical Co., U.K
Octane-1-sulfonic acid	Sigma Aldrich, Ireland
Serotonin (5-HT)	Sigma Chemical Co., U.K
Sodium phosphate monobasic monohydrate (NaH_2PO_4)	Sigma Aldrich, Ireland

2.1.4 MRI reagents

Gadolinium contrast agent (Magnevist)	Bayer Healthcare Pharmaceuticals, Germany

2.1.5 Evans blue assay reagents

Heparin sodium (Multiparin)	Bioresources, Trinity College Dublin
Trichloroacetic acid, 50%	Sigma Aldrich, Ireland
Ethanol, 100%	Sigma Aldrich, Ireland
Evans blue dye	Sigma Aldrich, Ireland

2.1.6 General Laboratory Chemicals

Paraformaldehyde	Sigma Aldrich, Ireland
Sodium chloride (NaCl)	BDH Chemicals, Poole, U.K.
Sodium hydroxide (NaOH)	Sigma Aldrich, Ireland
Sodium phosphate dibasic (NaH_2PO4)	Sigma Aldrich, Ireland
Sucrose	Sigma Aldrich, Ireland
Tween-20	Sigma Aldrich, Ireland

2.1.7 General Laboratory Plastics

Brain matrix	ASI Instruments, USA
Glass inserts	Fisher Scientific, Ireland
Glass screw top vials	Labquip Ltd., Ireland
Microtubes (1.5 ml)	Sarstedt, Ireland
Microscope slides	Fisher Chemical, U.K.
Parafilm laboratory rolls	Sarstedt, Ireland

Pipette tips	Sarstedt, Ireland
Black non-translucent 96 well plates	Thermo Scientific, Denmark

2.1.8 Anaesthetics

Ketamine (Narketan)	Bioresources, Trinity College Dublin
Xylazine (Chanazine)	Bioresources, Trinity College Dublin

2.2 Methods

2.2.1 Animals

Male Wistar rats (175 - 250 g) were obtained from the Bioresources Unit, Trinity College Dublin. Animals were housed in medium-sized, hard-bottomed propylene cages with stainless steel lids. Animals were housed 4 per cage under standard housing conditions at a constant temperature (20 ± 2°C) and at standard lighting conditions (12 hour light:12 hour dark cycle, lights on from 0800 to 2000 hours). Food and water were available *ad libitum*. The experimental protocol was carried out in accordance with the guidelines of the Animal Ethics Committee Trinity College Dublin and the European Council Directive 1986 (86/806/EEC).

2.2.2 Drug Preparation and Administration

All drugs were dissolved in saline (0.89% NaCl) and administered by the intraperitoneal (i.p.) route of injection, at an injection volume of 1 ml/kg. Metergoline was dissolved in Tween-saline (0.5% v/v).

2.2.3 Monitoring Body Temperature

Animals were lightly restrained by hand and core body temperature measurements were recorded using a lubricated digital rectal thermometer (Omron) inserted 3 cm into the

rectum. Temperature was recorded immediately prior to and every 30 min for up to 3 hr following drug administration.

2.2.4 Anaesthesia and Animal Preparation

Rats were anaesthetised with 0.1 - 0.2 ml ketamine (100 mg/ml) and 0.1 - 0.2 ml xylazine (20 mg/ml). Animals were subsequently placed onto a custom-built fibreglass cradle and temperature was maintained using a warming surface controlled by a water pump-driven temperature regulator (SA Instruments Inc., Stony Brook, NY, USA). A mechanical ventilator (Ugo Basile, Comerio, VA, Italy) was used to deliver adequate inflowing gas to the facemask and the respiration signal was monitored using custom hardware and software (SA Instruments Inc., Stony Brook, NY, USA). Animals were inserted into the centre of the 7 Tesla (7T), 30 cm bore animal MR system (Bruker Biospin 70/30 magnet system, Ettlingen, Germany). Anaesthetic depth was controlled by maintaining respiration rate in the range of 60 to 85 breaths per minute.

In some studies the right femoral vein was catheterised and used as a portal for administration of contrast agent. Briefly, an incision was made in the skin of the right inner thigh which exposed the femoral artery and vein. Forceps were used to separate the vein from the artery. Blood flow to the distal end of the vein was stopped using a cuff and a catheter was inserted. The catheter was fixed in place for administration of contrast agent. The partial pressure of carbon dioxide (pCO_2) and pH were measured, in a blood sample obtained from the catheterised femoral vein, using a calibrated transcutaneous blood gas analyser (TCM4, Radiometer Copenhagen, Willich, Germany) before commencing the scanning.

2.2.5 Magnetic Resonance Imaging

A high resolution anatomical scan (T_2-weighted RARE; Rapid Acquisition with Relaxation Enhancement) was generated using the following parameters: slice thickness = 1.5 mm, repetition time (TR) = 3134.511 ms, echo time (TE) = 12 ms, RARE factor = 8, RF flip angle = 90°/180°, field of view (FOV) = 3.0 x 3.0 cm, image matrix = 128 x 128, total scan time = 50 s.

A continuous ASL (cASL) sequence was subsequently applied, as previously described (Kelly et *al.*, 2009). Briefly, the sequence consisted of a 5 s preparation interval which contained the inversion pulse followed by snapshot fast low angle shot (FLASH) acquisition. The sequence was used to provide signal-time curves of the passage of a 3 s bolus through the primary motor cortex region. The following parameters were used: slice thickness = 2 mm, TR = 6.938 ms, TE = 2.63 ms, RF flip angle = 30°, FOV = 3.0 x 3.0 cm, image matrix = 128 x 64. Values for MTT, CTT, and signal amplitude were generated by fitting the non-compartmental model of cerebral perfusion to the experimental data (Kelly et *al.*, 2009). The cASL sequence was applied 6 times and signal averaging was performed to provide better signal to noise ratio.

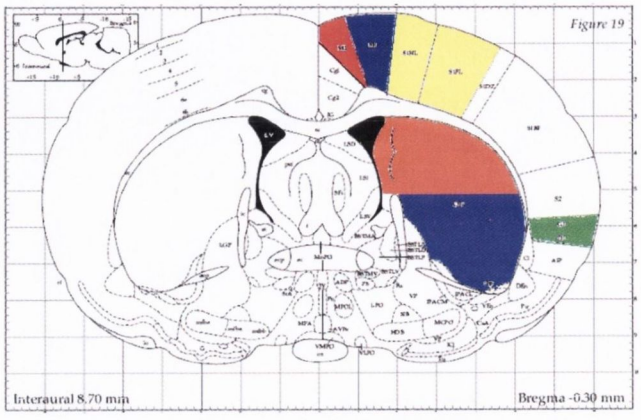
In the experiments with femoral vein catheterisation, a contrast-enhanced MRI approach was adopted for assessment of the integrity of the BBB. 5 min following completion of btASL, gadolinium (Magnevist) (1 ml; i.v.) was infused via the portal secured to the femoral vein. A FLASH scan sequence was employed to assess the entry and distribution of gadolinium throughout the cerebrovasculature. The following parameters were used: slice thickness = 1.2 mm, TR = 312.5 ms, TE = 2.53 ms, RF flip angle = 30°, FOV = 3.0 x 3.0

cm, image matrix = 128 x 128. Scanning continued for 55 min following gadolinium administration in order to allow sufficient time for the contrast agent to clear.

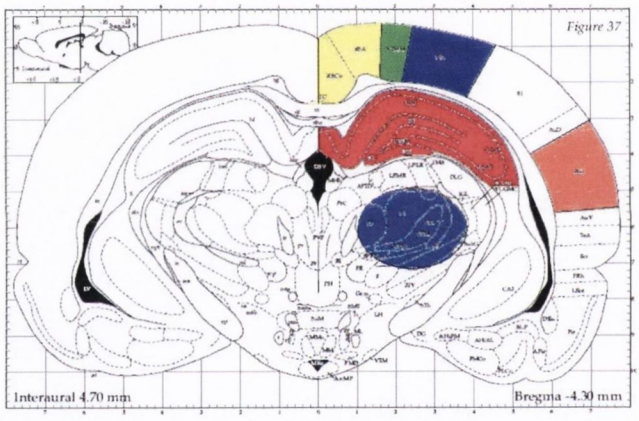
MRI data was analysed using the data acquisition and analysis software, Paravision (Bruker Biospin, Germany), and scripts written in Interactive Data Language (IDL; ITT Visual Informations Systems, USA) software version 7.0. An IDL 7.0 function was used to perform a subtraction of the labelled from the control images generated by the cASL sequence. ImageJ (Rasband, USA) software was used to select regions of interest (ROI), within two coronal slices, for analysis. A signal-time curve was generated for each ROI. The curve-fitting routine in Mathematica (Wolfram Research Inc, Version 5.1, Champaign, IL, USA) was used to calculate the MTT, CTT and signal amplitude parameters from the btASL signal-time curves. In addition to the inbuilt functions of IDL, use was also made of the Coyote IDL Library (Fanning Software Consulting, USA; downloaded from http://www.dfanning.com) to generate CBV maps. Changes in CBV may be represented on a colour scale adjacent to the CBV maps. Brighter colours on the CBV scale indicate areas of increased CBV with the brightest areas corresponding to those ROIs with highest CBV. ImageJ was used to select ROIs for analysis. Data from these regions were analysed using IDL scripts to generate CBV maps.

ROIs were drawn in the spatially normalised high resolution anatomical brain image obtained for each subject. Analysis of two brain sections at different levels along the coronal plane was carried out. The first coronal section comprised motor, somatosensory and insular cortex as well as striatum. The second coronal section chosen comprised visual, auditory, parietal association and retrosplenial cortex in addition to thalamus and hippocampus.

Regions of Interest used for btASL MRI analysis



- Primary Motor Cortex
- Secondary Motor Cortex
- Primary Somatosensory Cortex
- Insular Cortex
- Dorsal Striatum
- Ventral Striatum



- Retrosplenial Cortex
- Visual Cortex
- Parietal Association Cortex
- Auditory Cortex
- Hippocampus
- Thalamus

Modified from Paxinos and Watson Brain Atlas, 1998

2.2.6 Test for Extravasation of Evans Blue

As gadolinium enhanced MRI has not been employed to determine drug-induced changes to BBB integrity in animals previously, extravasation of Evans blue dye was assessed in separate groups of animals treated identically to those undergoing MR imaging. 3 hr following MDMA (20 mg/kg; i.p.) administration, animals were anaesthetised and the right femoral vein was catheterised as previously described. Evans blue dye (2 % solution in 0.89 % saline; 0.3 ml/100 g body weight) was infused via the femoral vein portal and allowed to circulate for 5 min. Following this, the thoracic cavity was opened and the animals were transcardially perfused. A small incision was made at the apex of the left ventricle and a gavage was inserted into the aorta to ensure complete perfusion of the systemic circulation. A right atrial incision was also made to prevent blood from re-entering the systemic circulation. Heparinised saline (0.05% v/v) was infused through the gavage over a 5 min period followed by paraformaldehyde (4% in phosphate buffered saline (PBS)) over 10 min (flow rate of 18 ml/min). Animals were decapitated and perfused brains were dissected free and post-fixed in paraformaldehyde (4% in PBS) for 24 hr followed by immersion in a cryoprotectant sucrose solution (30% in PBS) for 48 hr. 3 mm coronal sections of perfused brain were prepared using a brain matrix (RBM 400C; ASI Instruments, USA) and photographed (Canon EOS 5D) to enable macroscopic inspection for blue discolouration associated with the presence of the dye in the brain parenchyma. Brain regions were subsequently dissected from the sections, weighed, homogenised (Branson Sonifier 150) in 2 volumes of 50% trichloroacetic acid (TCA) (1 volume = 1g/1ml) and centrifuged (Eppendorf Centrifuge 5415R) for 20 min at 10,000 rpm at 4°C. Supernatant was diluted 1:3 with 100% ethanol and assayed for the presence of Evans blue dye. A set of standards of known Evans Blue dye concentration (0 - 1000 ng/ml) were

prepared in 50% TCA:ethanol (1:3). Standards and samples were plated out onto black non-translucent 96 well plates in duplicate and the plates were read on a fluorescence spectrophotometer (FLUOstar Optima, BMG Labtech) using excitation and emission wavelengths of 544 nm and 650 nm, respectively. The quantity of Evans blue dye (µg/mg fresh tissue) extracted from the brain samples was determined using the standard curve.

2.2.7 High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) mobile phase (100 mM citric acid, 100 mM NaH_2PO_4, 1.4 mM octane-1-sulfonic acid, 100 mM EDTA, 10% (v/v) methanol) was prepared using double-distilled, re-filtered NANOpure H_2O. pH of the solution was adjusted to 2.8 using 4M NaOH and the mobile phase was vacuum filtered. Standards of 5-HT, 5-HIAA and *N*-methyl-5-HT were prepared as standard calibration points and to assess retention times on the HPLC column. Standards were dissolved in 10 ml HPLC mobile phase to give solutions of 1 mg/ml and these were used to prepare standard mixture containing a final concentration of 5 ng/20 µl of each standard in HPLC mobile phase.

Tissue samples were weighed and suspended in 0.5ml ice-cold homogenisation buffer (HPLC mobile phase containing 5 ng/20 µl *N*-methyl-5-HT as an internal standard). Samples were homogenised by sonication (MSE sonicator) for 5 - 10 s, centrifuged (Eppendorf Centrifuge 5415R) for 15 min at 13,000 g at 4°C and supernatant was filtered to remove any remaining protein debris. 150 µl supernatant was transferred into an insert within a vial and air bubbles, if present, were removed by tapping. Vials containing 150 µl standard mixture were placed after every 5 samples. Samples and standards were analysed using an automated HPLC system (Shimadzu ADVP module) and an autosampler injected 10 µl into the HPLC reverse phase

column (Kinetex™ Core Shell Technology column with specific area of 100mm x 4.6mm and particle size of 2.6u, Phenomenex). 5-HT and 5-HIAA concentration was quantified by electrochemical detection (Antec Decade, +0.8V) and chromatograms were generated (Class VP software integration). Peak heights of 5-HT, 5-HIAA and internal standard were used to calculate (ng/g fresh tissue) brain biogenic amine concentration.

2.2.8 Statistical Analysis

Statistical analysis was carried out using GB-Stat v.10. One-way, two-way and repeated measures analyses of variance (ANOVA) were performed where appropriate. If significant changes were observed, Fisher's LSD or Student Newman-Keuls *post hoc* test was carried out. Comparisons between two isolated groups were carried out by Student's *t*-test. Differences between groups were deemed significant when $p<0.05$. Results are expressed as mean with standard error of the mean (SEM).

Chapter 3

Regional, time and dose dependent effects of MDMA "Ecstasy" on cerebral perfusion determined by bolus-tracking arterial spin labelling (btASL) MRI

Chapter 3: Regional, time and dose dependent effects of MDMA "Ecstasy" on cerebral perfusion determined by bolus-tracking arterial spin labelling (btASL) MRI

3.1 Introduction

There are numerous case studies that have linked recreational MDMA ("Ecstasy") use to the incidence of cerebrovascular events including cerebral infarction, subarachnoid haemorrhage and intracerebral haemorrhage (De Silva & Harries, 1992; Gledhill *et al.*, 1993; Hanyu *et al.*, 1995; Harries & De Silva, 1992; Henry, 1992; Henry *et al.*, 1992; Hughes *et al.*, 1993; McEvoy *et al.*, 2000; Reneman *et al.*, 2000, for review; Teggin, 1992). Awareness is growing of the increased incidence of cerebrovascular accidents in young people with a history of exposure to recreational drugs in general and MDMA in particular (Agaba *et al.*, 2002; Auer *et al.*, 2002; Ferrington *et al.*, 2006; Gledhill *et al.*, 1993; Hanyu *et al.*, 1995; Kaku & Lowenstein, 1990; Miranda & O'Neill, 2002; Perez *et al.*, 1999; Petitti *et al.*, 1998). The mechanisms underlying such effects are unclear. However, a role for 5-HT is implicated on account of the acute effects of MDMA on 5-HT release from serotonergic nerve endings and the proposed involvement of 5-HT in the regulation of the brain microcirculation, with perivascular serotonergic innervations providing vasoconstrictor tone throughout the cerebrovasculature, from the major arteries to the intraparenchymal resistance vessels (Cohen *et al.*, 1996; Parsons, 1991), suggesting a role for 5-HT in the regulation of brain microcirculation. As cerebral microvessels are innervated by 5-HT having mixed but mainly vasoconstrictive actions, altered 5-HT innervation to cerebral blood vessels following MDMA exposure may produce changes in resting vascular tone.

The presence of reduced blood flow following MDMA administration in humans was first reported by Chang and colleagues (2000) who evaluated changes to CBF with both ^{133}Xe and ^{99m}Tc-hexamethylpropyleneamine oxime (HMPAO) single photon emission computerised tomography (SPECT) co-registered with MRI. Abstinent MDMA users were given oral dosages of MDMA (2.25 - 4.75 mg/kg) on two occasions 1 to 3 wk apart and studied 2 to 3 wk following the second dose. Significant CBF reductions in multiple brain regions including the bilateral caudate, bilateral superior parietal cortex and right dorsolateral prefrontal cortex were observed in these subjects when compared to matched controls. The largest reductions in CBF were noted in the parietal and dorsolateral frontal brain regions which receive extensive 5-HT innervations (Cohen *et al.*, 1996). These findings suggested a sub-chronic persistent vasoconstrictive effect of MDMA administration leading investigators to speculate that long-lasting changes to 5-HT function might underlie the observed effects (Chang *et al.*, 2000). Similar conclusions were drawn in a HMPAO SPECT investigation of a 19 year old woman who, 20 days following ecstasy intoxication, showed decreased cortical blood flow (Finsterer *et al.*, 2003). The authors proposed that this impairment may be explained by vasoconstriction following ecstasy-induced changes to 5-HT transmission.

In a $H_2{}^{15}O$-positron emission tomography (PET) study of 16 drug naïve humans, MDMA (1.6 mg/kg; p.o.), a dose lower than that reported in the Chang study, increased CBF was measured in diverse regions including the ventromedial frontal and occipital cortex, inferior temporal lobe and cerebellum cortex. Decreases in CBF were recorded in the motor and somatosensory cortex, temporal lobe including left amygdala, cingulate and insular cortex and thalamus (Gamma *et al.*, 2000). PET measurements were started 75 min after drug intake, at the time of peak drug effects (Gamma *et al.*, 2000; Vollenweider *et al.*, 1998).

Using CBV maps calculated from dynamic susceptibility contrast (DSC)-MRI, Reneman and co-workers (2000) have previously reported lower relative CBV (rCBV) in the globus pallidus and occipital cortex in recent MDMA users (subjects having a mean abstinence period of 7 wk) when compared to non-users. A positive correlation between 5-HT_{2A} receptor densities, measured with the high affinity 5-HT_{2A} receptor ligand [^{123}I]R91150 SPECT, and rCBV was found in the occipital cortex and globus pallidus of MDMA users but not in control subjects. The changes were reported to be associated with 5-HT release, stimulation of 5-HT_2 receptors leading to vasoconstriction and subsequent down-regulation of 5-HT_2 receptors, on the vasculature. Both are regions that receive innervation from the 5-HT system and may be particularly sensitive to 5-HT neuronal injury following exposure to MDMA (Scheffel *et al.*, 1998; Spatt *et al.*, 1997; Squier *et al.*, 1995).

de Win and colleagues (2007) prospectively studied sustained effects (> 2 wk abstinence) of a low dose of ecstasy (1.8 ± 1.3 tablets) on the brain in ecstasy naïve volunteers using DSC-MRI for the determination of rCBV. A sustained decrease in rCBV in the thalamus, dorsolateral frontal cortex and superior parietal cortex was observed in new low dose ecstasy users. In line with previous investigations the authors propose that decreases in CBV may indicate that even low ecstasy doses can induce prolonged vasoconstriction in some brain areas due to sustained MDMA mediated serotonergic effects. Thus, taking clinical investigations carried out in drug users to date it is proposed that ecstasy use induces a sub-acute increase of extracellular 5-HT leading to vasoconstriction which subsequently leads to reduced CBF and CBV particularly in areas receiving 5-HT innervation.

There are several potential limitations of conclusions drawn from such investigations on recreational drug users including the retrospective nature of the study design which fails to address the possibility of pre-existing differences between drug users and non-users as users may be predisposed to have lower CBV values. Furthermore, drug users are likely to experiment with other recreational drugs and although participants abstain from use of MDMA before the study, the possibility remains that effects observed may be at least partially attributable to the actions of other psychoactive substances. In order to gain greater insight and clarity with regard to the acute, sub-acute and chronic effects of MDMA, animal studies investigating CBF and CBV changes following MDMA administration are of importance. In this regard it has been previously shown in laboratory animal investigations that MDMA-induced 5-HT dysfunction alters cerebrovascular control mechanisms in a manner that is consistent with the known vasoconstrictor properties of 5-HT (Ferrington *et al.*, 2006; Quate *et al.*, 2004).

The earliest of such investigations include a study carried out by Kelly and co-workers (1994) where, using a quantitative autoradiographic approach with [^{14}C] iodoantipyrine and [^{14}C]-2-deoxyglucose, local cerebral blood flow (LCBF) and cerebral glucose utilisation (LCMRglu) respectively were measured in rat neocortex, hippocampus and striatum following MDMA administration (5 mg/kg; i.v.). In control rats, CBF was coupled to CMRglu, but in MDMA-treated rats, marked hyperperfusion was measured in frontal and parietal cortex with no change in glucose utilisation. This suggests that MDMA has the potential to disrupt the regulation of cerebrovascular tone (Kelly *et al.*, 1994).

Quate *et al.*, (2004), using the same approach, subsequently investigated LCBF changes in Dark Agouti rats following MDMA (15mg/kg; i.p.) administration. Whereas frontal cortex

and globus pallidus displayed increased LCBF, a large number of regions including limbic areas displayed decreased LCBF 25 min following MDMA administration. By contrast MDMA produced significant increases in LCMRglu in 28 brain areas amongst 50 tested and most markedly in the motor system including the globus pallidus. The results provided evidence for the uncoupling of LCBF from underlying metabolic demand, possibly due to the vasoconstrictive action of 5-HT (Quate *et al.*, 2004). The regional differences reported in response to MDMA imply that the frontal cortex is subject to actions which oppose those found in other areas of the brain. Increased LCBF observed in the frontal cortex in the absence of any change in LCMRglu was indicative of a hyperaemic response in this region of the brain.

In a follow-up investigation, Ferrington and co-workers (2006) reported that MDMA (15 mg/kg) administered to Dark Agouti rats produced significant increases in LCMRglu in 16 of the 44 brain areas analysed and most notably in the motor system but also including regions within the somatosensory and limbic systems. LCBF was significantly decreased in 4 of 44 brain regions analysed including the lateral and medial habenula, the posterior cingulate cortex and the anterior thalamus when compared to saline treated controls. All drug effects on LCBF were reported 25 min following drug administration. The authors proposed that the results were consistent with MDMA-induced perivascular release of 5-HT mediating vasoconstriction, independent of and opposed to the dilatory drive from increased metabolism. Acute MDMA-induced decreases in tissue perfusion, despite a marked increase in cerebral metabolic demand, were proposed to represent a state of unstable oligemia potentially damaging to the surrounding tissue. In only 4 areas of the brain, all in the neocortex, were the effects of acute MDMA treatment upon LCBF qualitatively similar to the increases observed in LCMRglu. The effects observed in the

neocortex were reminiscent of those reported by Quate *et al.*, (2004) in the frontal cortex under similar experimental conditions.

Using an alternative technique and species, in a PET study with statistical parametric mapping, Rosa-Neto and co-workers (2004) undertook an analysis of focal changes in CBF in the anaesthetised female Landrace pig 30 and 150 min following acute administration of MDMA (1mg/kg; i.v.). Increases in CBF were reported 30, but not 150, min following MDMA administration, in occipital cortex but no further regions. It is noteworthy that an earlier PET study in MDMA naïve human subjects also reported drug-induced increases in CBF in ventromedial frontal and occipital cortex in addition to decreases in other regions as discussed previously (Gamma *et al.*, 2000). The results however were consistent with previous reports of increased CBF in cortical fields associated with acute MDMA administration.

When taken together it is clear that MDMA is associated with both increases and decreases in CBF and CBV which are time, dose and region-dependent. Given the potential for 5-HT to induce cerebrovascular constriction it is somewhat surprising that MDMA can also provoke increases in CBF, which is likely related to vasodilatation and mediated by other neurotransmitters such as dopamine or alternative CNS mediators. A further contributing factor however may also relate to changes in the permeability of the blood brain barrier (BBB). Sustained increases in BBB permeability have previously been reported following MDMA administration to rats (Bankson, 2005). Administration of MDMA (10 mg/kg; i.p., 4 times daily) resulted in increased extravasation of trypan blue dye and consequent staining of coronal sections in comparison to vehicle treated controls. Significant increases in staining were reported at the level of the caudate both 24 hr and 10 wk after treatment with MDMA. In a subsequent investigation Sharma & Ali (2008) demonstrated that a

single high dose of MDMA (40 mg/kg; i.p.) administered to rats and mice resulted in the extravasation of Evans blue dye in the cerebral cortex, hippocampus, cerebellum, and thalamus brain 4 hr post drug administration. In addition mild to moderate extravasation was observed in the walls of the lateral and fourth ventricles indicating that in addition to BBB disruption there was also a reduction in integrity of the blood-cerebrospinal fluid barrier. Consideration therefore should be given to a potential role for BBB disruption as a contributing factor in mediating MDMA-induced changes in CBF or CBV.

Recently a new quantitative bolus-tracking arterial spin labelling (btASL) MRI technique was developed and described by Kelly and colleagues (2009) for the measurement of perfusion state in the rodent brain. The technique assesses cerebral perfusion through the calculation of two transit times: the mean transit time (MTT) which represents the time taken for labelled arterial blood to traverse the vasculature and reach the imaging plane and the capillary transit time (CTT) which represents the time taken for the blood to disperse at the imaging plane. MTT is inversely proportional to CBF, while CTT is inversely proportional to CBF squared. A third quantifiable output is the signal amplitude, which is derived from the area under the btASL signal-time curve and has been interpreted as being proportional to CBV.

The objective of this investigation was to use the btASL technique to determine regional, time and dose-dependent changes to cerebral perfusion in the rat following single acute administration of MDMA. In addition it was deemed necessary to clarify if any changes observed were associated with BBB disruption.

3.2 Experimental Procedure

Study 1

MDMA-induced changes in CBF and CBV were examined over time. Animals received a single administration of MDMA (20 mg/kg; i.p.) or vehicle (saline) and were placed into the MRI scanner 3 or 24 hr later. BBB integrity was assessed *in vivo* and *ex vivo* in the 3 and 24 hr treatment groups. In a separate experiment where the femoral vein was not prepared for gadolinium infusion, animals received MDMA (20 mg/kg; i.p.) or vehicle (saline) and were placed into the MRI scanner 1 hr later. This was carried out to capture the effects of MDMA at an earlier time following drug administration.

Study 2

Dose-dependent changes in cerebral perfusion following MDMA administration were examined. Animals received a single administration of MDMA (5 or 20 mg/kg; i.p.) and were placed into the scanner 3 hr later.

3.3 Results

pCO_2 was recorded as 44.86 ± 1.39 mm Hg and 46.64 ± 1.57 mm Hg; pH as 7.3 ± 0.01 and 7.39 ± 0.05 and maximum core body temperature increase as 2.01°C ± 0.16°C and 0.38°C ± 0.18°C in MDMA (20mg/kg; i.p., n=16) and vehicle treated animals (saline, n=16), respectively.

3.3.1 MDMA provokes a time dependent decrease in MTT and CTT and an increase in signal amplitude in the primary motor cortex

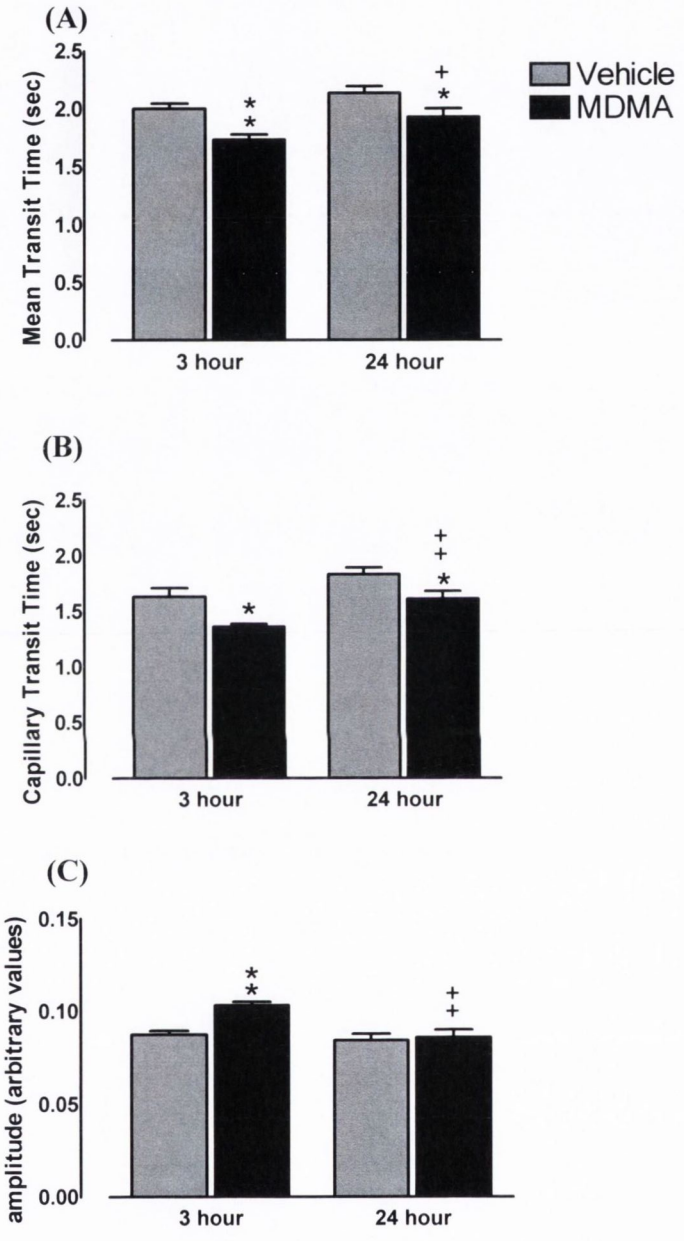
Primary Motor Cortex

(A) MTT: ANOVA showed an effect of time [$F_{(1,28)}$=9.68, p<0.05] and an effect of MDMA [$F_{(1,28)}$=17.413, p<0.001]. *Post hoc* comparisons revealed that MTT was decreased in MDMA treated animals 3 and 24 hr (p<0.01) following drug administration when compared to vehicle treated controls. There was an increase in MTT over time when MDMA treated animals were compared 3 and 24 hr following drug administration (p<0.05) (Figure 3.3.1.1 (A)). Student's *t*-test revealed a significant reduction in MTT 1 hr (p<0.001) following drug administration when compared to vehicle treated controls (1.84 ± 0.04 and 1.55 ± 0.05, respectively) (data not shown).

(B) CTT: ANOVA showed an effect of time [$F_{(1,28)}$=15.32, p<0.001] and an effect of MDMA [$F_{(1,28)}$=17.143, p<0.001]. *Post hoc* comparisons revealed that CTT was decreased in MDMA treated animals 3 and 24 hr (p<0.05) following drug administration when compared to vehicle treated controls. There was an increase in

CTT over time when MDMA treated animals were compared 3 and 24 hr following drug administration ($p<0.05$) (Figure 3.3.1.1 (B)). Student's *t*-test revealed a significant reduction in CTT 1 hr ($p<0.01$) following drug administration when compared to vehicle treated controls (1.55 ± 0.06 and 1.29 ± 0.03, respectively) (data not shown).

(C) Signal amplitude: ANOVA showed an effect of time [$F_{(1,28)}=12.08$, $p<0.01$] and an effect of MDMA [$F_{(1,28)}=10.69$, $p<0.01$]. *Post hoc* comparisons revealed that signal amplitude was increased in MDMA treated animals 3 ($p<0.01$) but not 24 hr following drug administration when compared to vehicle treated controls. There was a decrease in signal amplitude over time in MDMA treated animals when compared 3 and 24 hr following drug administration ($p<0.01$) (Figure 3.3.1.1 (C)). Student's *t*-test revealed a significant increase in signal amplitude 1 hr ($p<0.01$) following drug administration when compared to vehicle treated controls (0.09 ± 0.002 and 0.11 ± 0.044, respectively) (data not shown).


(A)
Mean Transit Time (sec)
2.5
2.0
1.5
1.0
0.5
0.0
Vehicle
MDMA
3 hour
24 hour
(B)
Capillary Transit Time (sec)
2.5
2.0
1.5
1.0
0.5
0.0
3 hour
24 hour
(C)
amplitude (arbitrary values)
0.15
0.10
0.05
0.00
3 hour
24 hour

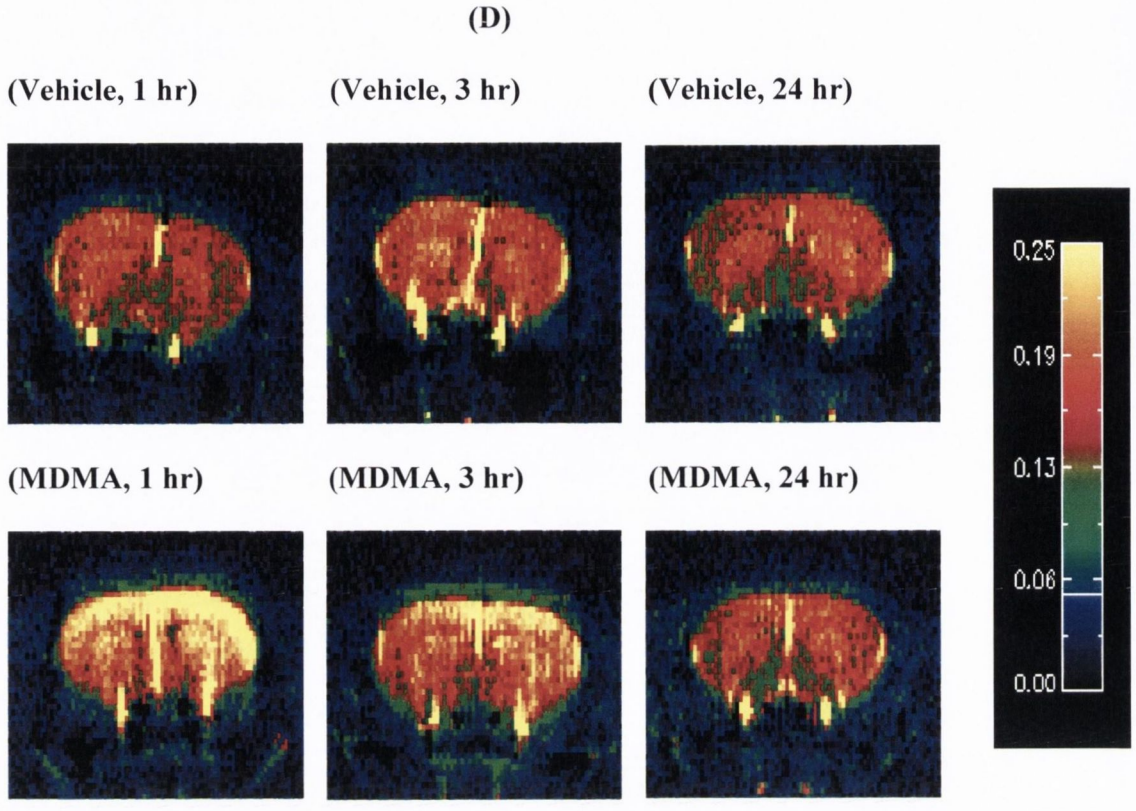


Figure 3.3.1.1 MDMA provokes a time dependent decrease in MTT and CTT with a corresponding increase in signal amplitude in the primary motor cortex

MDMA (20 mg/kg) provokes a decrease in (A) MTT and (B) CTT 3 and 24 hr following drug administration and increases (C) signal amplitude 3 hr following drug administration when compared to vehicle treated controls in the primary motor cortex.

(D) CBV maps depicting MDMA-induced increases in CBV 1 and 3 hr following drug administration in a representative coronal brain slice. Colour change depicting changes to CBV had largely returned to baseline, being indistinguishable from vehicle control, 24 hr following drug administration. Data are expressed as mean ± SEM (n=8). * $p< 0.05$; ** $p<0.01$ vs. vehicle at corresponding time point. + $p<0.01$ vs. MDMA (3 hr) (Student Newman-Keuls *post hoc* test).

Secondary Motor Cortex

MDMA provoked a decrease in MTT and CTT and an increase in signal amplitude in the secondary motor cortex 3 but not 24 hr following drug administration when compared to vehicle treated controls (Table 3.3.1).

(A) MTT: ANOVA showed an effect of MDMA [$F_{(1,28)}$=7.86, p<0.01]. *Post hoc* comparisons revealed that MTT was decreased in MDMA treated animals 3 hr (p<0.05) following drug administration when compared to vehicle treated controls.

(B) CTT: ANOVA showed an effect of time [$F_{(1,28)}$=5.57, p<0.05] and an effect of MDMA [$F_{(1,28)}$=9.55, p<0.01]. *Post hoc* comparisons revealed that CTT was decreased in MDMA treated animals 3 hr (p<0.05) following drug administration when compared to vehicle treated controls. There was an increase in CTT over time when MDMA treated animals were compared 3 and 24 hr following drug administration (p<0.05).

(C) Signal amplitude: ANOVA showed an effect of time [$F_{(1,28)}$=6.76, p<0.05] and an effect of MDMA [$F_{(1,28)}$=6.31, p<0.05]. *Post hoc* comparisons revealed that signal amplitude was increased in MDMA treated animals 3 (p<0.01) but not 24 hr following drug administration when compared to vehicle treated controls. There was a decrease in signal amplitude over time in MDMA treated animals when compared 3 and 24 hr (p<0.05) following drug administration.

Decreases in MTT (p<0.01) and CTT (p<0.05) and an increase in signal amplitude (p<0.05) were evident 1 hr following MDMA administration in secondary motor cortex in

comparison to vehicle treated controls (1.97 ± 0.07 and 1.64 ± 0.07, 1.65 ± 0.1 and 1.34 ± 0.05 and 0.092 ± 0.003 and 0.105 ± 0.004, respectively).

Somatosenory Cortex

MDMA provoked a decrease in MTT and CTT and an increase in signal amplitude in the somatosensory cortex 3 but not 24 hr following drug administration when compared to vehicle treated controls (Table 3.3.1).

(A) MTT: ANOVA showed an effect of time [$F_{(1,28)}$=15.63, p<0.001], an effect of MDMA [$F_{(1,28)}$=23.32, p<0.001] and a time x MDMA interaction effect [$F_{(1,28)}$=8.35, p<0.01]. *Post hoc* comparisons revealed that MTT was decreased in MDMA treated animals 3 hr (p<0.01) following drug administration when compared to vehicle treated controls. There was an increase in MTT over time when MDMA treated animals were compared 3 and 24 hr (p<0.01) following drug administration.

(B) CTT: ANOVA showed an effect of time [$F_{(1,28)}$=17.46, p<0.001], an effect of MDMA [$F_{(1,28)}$=20.25, p<0.001] and a time x MDMA interaction effect [$F_{(1,28)}$=4.59, p<0.05]. *Post hoc* comparisons revealed that CTT was decreased in MDMA treated animals 3 hr (p<0.01) following drug administration when compared to vehicle treated controls. There was an increase in CTT over time when MDMA treated animals were compared 3 and 24 hr (p<0.01) following drug administration.

(C) Signal amplitude: ANOVA showed an effect of time [$F_{(1,28)}$=25.23, p<0.001], an effect of MDMA [$F_{(1,28)}$=11.64, p<0.01] and a time x MDMA interaction effect [$F_{(1,28)}$=11.622, p<0.01]. *Post hoc* comparisons revealed that signal amplitude was increased in MDMA treated animals 3 (p<0.01) but not 24 hr following drug administration when compared to vehicle treated controls. There was a decrease in signal amplitude over time in MDMA treated animals when compared 3 and 24 hr (p<0.01) following drug administration.

Decreases in MTT (p<0.001) and CTT (p<0.01) and an increase in signal amplitude (p<0.01) were evident 1 hr following MDMA administration in somatosensory cortex in comparison to vehicle treated controls (1.69 ± 0.04 and 1.42 ± 0.04, 1.52 ± 0.07 and 1.24 ± 0.03 and 0.098 ± 0.003 and 0.12 ± 0.005, respectively).

Other regional effects

A reduction in MTT and CTT and increase in signal amplitude (p<0.05; Student's t-test) was observed in the ventral striatum 1 hr following MDMA administration when compared to vehicle treated controls (1.66 ± 0.03 and 1.57 ± 0.02, 1.39 ± 0.04 and 1.31 ± 0.01 and 0.098 ± 0.002 and 0.104 ± 0.002, respectively) however, no effects of MDMA were observed in this region 3 or 24 hr following drug administration.

An increase in signal amplitude in the absence of significant changes in transit times was observed in the auditory cortex (p<0.001; 0.076 ± 0.001 and 0.095 ± 0.0024), parietal association cortex (p<0.01; 0.073 ± 0.003 and 0.099 ± 0.004) and thalamus (p<0.05; 0.084 ± 0.002 and 0.097 ± 0.004) 3 hr, but at no other time, following MDMA administration in comparison to vehicle treated controls.

No further changes in MTT, CTT or signal amplitude were observed in dorsal striatum, retrosplenial cortex, visual cortex or hippocampus following MDMA administration at any of the times assessed (data not shown).

Table 3.3.1 MDMA provokes a time dependent decrease in MTT and CTT with a corresponding increase in signal amplitude in cortex

		3 hr			24 hr		
		MTT (s)	*CTT (s)*	*Amplitude(a.u.)*	*MTT (s)*	*CTT (s)*	*Amplitude(a.u.)*
Secondary Motor	**Vehicle**	2.15 ± 0.06	1.77 ± 0.08	0.08 ± 0.002	2.19 ± 0.06	1.82 ± 0.07	0.08 ± 0.004
	MDMA	1.84 ± 0.04 *	1.41 ± 0.02 *	0.1 ± 0.003 **	2.08 ± 0.07	1.72 ± 0.09 +	0.08 ± 0.005 +
Somatosensory	**Vehicle**	1.84 ± 0.06	1.56 ± 0.07	0.09 ± 0.003	1.92 ± 0.03	1.66 ± 0.05	0.088 ± 0.003
	MDMA	1.57 ± 0.07 **	1.3 ± 0.03 **	0.114 ± 0.004 **	1.77 ± 0.06 ++	1.52 ± 0.07 ++	0.088 ± 0.004 ++

Data are expressed as mean ± SEM (n=8). * $p<0.05$ and ** $p<0.01$ vs. vehicle at corresponding time point. + $p<0.05$ and ++ $p<0.01$ vs. MDMA (3 hr) (Student Newman-Keuls *post hoc* test).

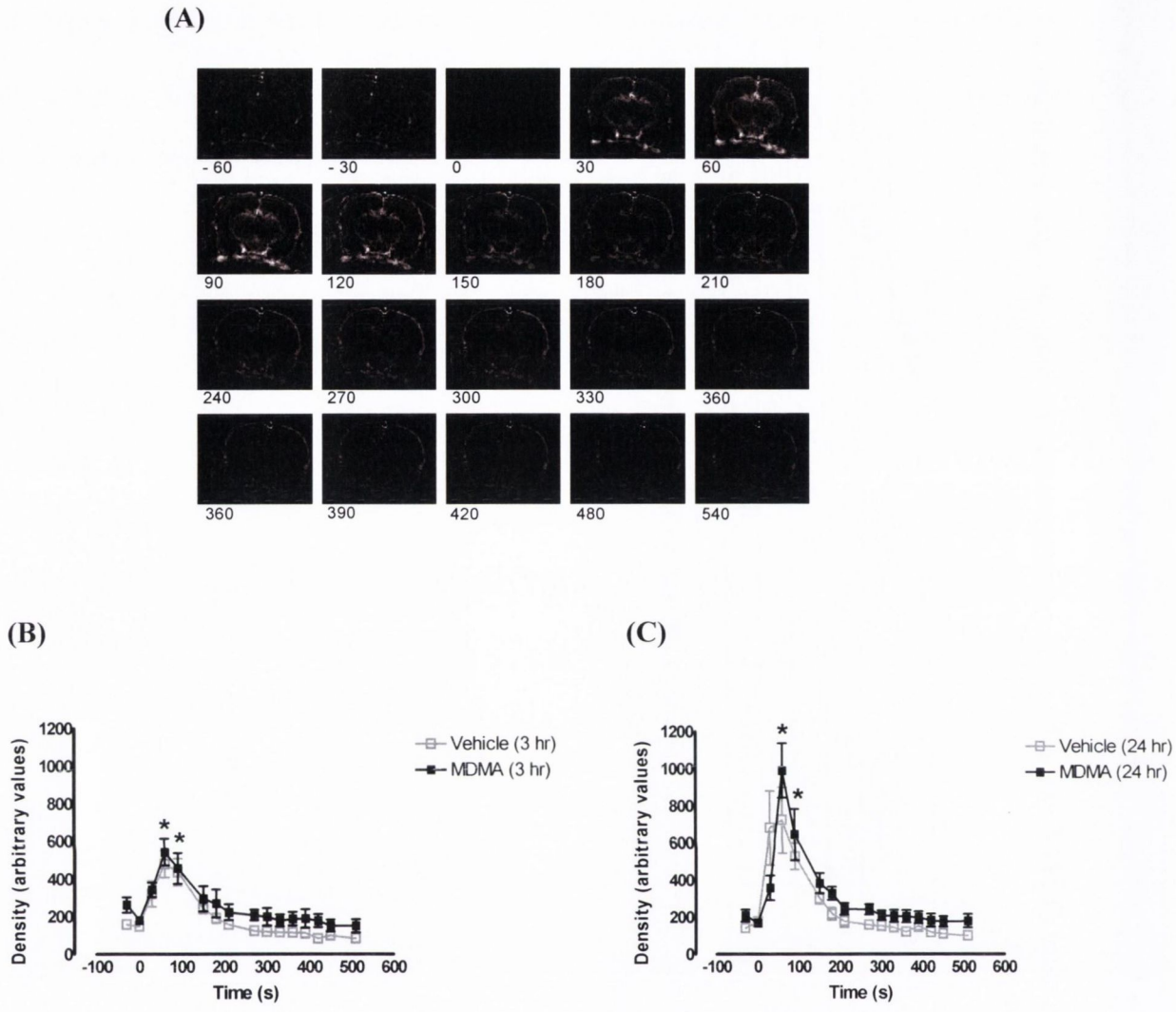


Figure 3.3.1.2 MR images representing the appearance and clearance of Gadolinium contrast agent over time following intravenous administration

Images show the appearance of gadolinium in a 30 s time series pre- and post-intravenous administration of gadolinium. The images (A) were generated by subtraction of background (time 0) prior to and following infusion. Analysis of change in density showed an increase in contrast that peaks after 1 min followed by a return to baseline 3 min later. No differences in contrast change were found between MDMA and vehicle treated animals either 3 (B) or 24 (C) hr after drug administration. Data are expressed as mean density ± SEM (n=8).

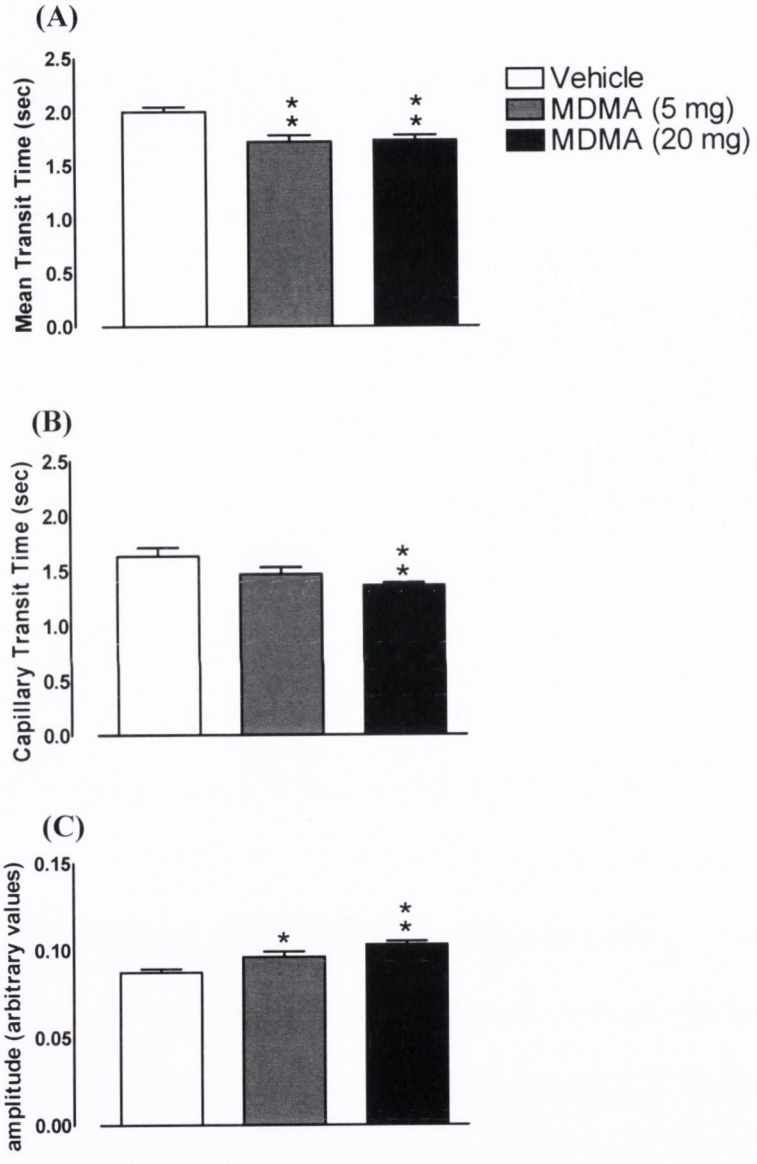
3.3.2 MDMA provokes a dose-dependent decrease in MTT and CTT with a corresponding increase in signal amplitude in the primary motor cortex

Primary Motor Cortex

(A) MTT: ANOVA showed an effect of MDMA [$F_{(2,21)}$=10.4, p<0.001]. *Post hoc* comparisons revealed that MTT was decreased 3 hr following MDMA (5 and 20 mg/kg) administration when compared to vehicle treated controls (p<0.01). The magnitude of change in MTT was similar with both doses of MDMA (Figure 3.3.2 (A)).

(B) CTT: ANOVA showed an effect of MDMA [$F_{(2,21)}$=5.38, p<0.05]. *Post hoc* comparisons revealed dose-dependent effects where CTT was reduced 3 hr following MDMA (20 mg/kg) administration only when compared to vehicle treated controls (p< 0.05) (Figure 3.3.2 (B)).

(C) Signal amplitude: ANOVA showed an effect of MDMA [$F_{(2,21)}$=10.32, p<0.001]. *Post hoc* comparisons revealed a dose-dependent increase in signal amplitude 3 hr following MDMA administration (5 and 20 mg/kg) when compared to vehicle treated controls (p<0.05; p<0.01 respectively). The magnitude of change in amplitude was greater with the higher dose of MDMA (Figure 3.3.2 (C)).


(A)
Mean Transit Time (sec)
2.5
2.0
1.5
1.0
0.5
0.0
**
**
Vehicle
MDMA (5 mg)
MDMA (20 mg)
(B)
Capillary Transit Time (sec)
2.5
2.0
1.5
1.0
0.5
0.0
**
(C)
amplitude (arbitrary values)
0.15
0.10
0.05
0.00
*
**

(D)

(Vehicle) **(MDMA, 5 mg/kg)** **(MDMA, 20 mg/kg)**

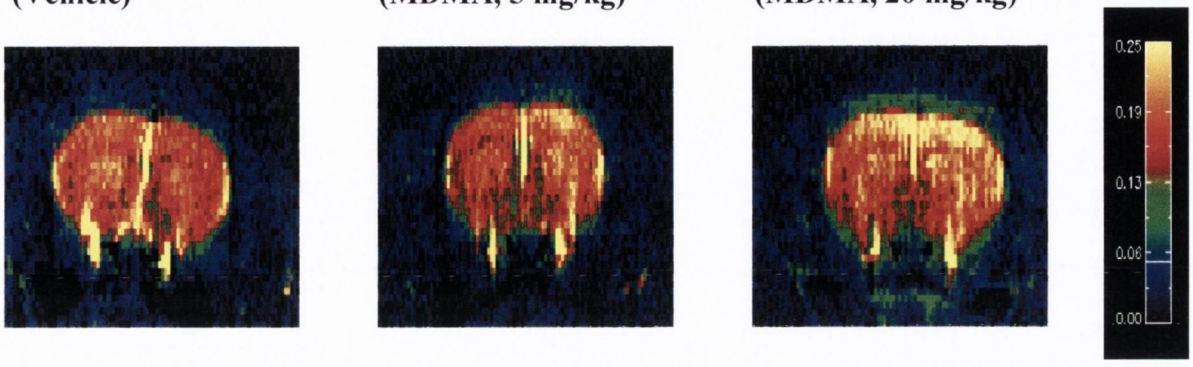


Figure 3.3.2 Dose-dependent decreases in MTT and CTT and increase in signal amplitude in the primary motor cortex

MDMA (5 and 20 mg/kg) induced a decrease in (A) MTT and corresponding increase in (C) signal amplitude in primary motor cortex. MDMA (20 mg/kg) induced a decrease in (CTT). The effects of MDMA on CTT and amplitude were dose-dependent. (D) CBV maps depicting dose-dependent MDMA-induced increases in CBV 3 hr following drug administration in a representative coronal brain slice. Increased CBV is evident in cortical regions following administration of MDMA (5 and 20 mg/kg) in comparison to a vehicle treated control animal. Data are expressed as mean ± SEM (n=8). * $p<0.05$; ** $p<0.01$ vs. vehicle (Dunnett's *post hoc* test).

Secondary Motor Cortex

MDMA provoked a dose-dependent decrease in MTT, CTT and increase in signal amplitude in secondary motor cortex 3 hr following drug administration when compared to vehicle treated controls (Table 3.3.2).

(A) MTT: ANOVA showed an effect of MDMA [$F_{(2,21)}$=2.93, p<0.05]. *Post hoc* comparisons revealed that MTT was decreased 3 hr following MDMA (20 mg/kg) administration when compared to vehicle treated controls (p<0.05). The magnitude of change in MTT was similar with both doses of MDMA.

(B) CTT: ANOVA showed an effect of MDMA [$F_{(2,20)}$=7.68, p<0.01]. *Post hoc* comparisons revealed dose-dependent effects where CTT was reduced 3 hr following MDMA (20 mg/kg) administration only when compared to vehicle treated controls (p<0.01).

(C) Signal amplitude: ANOVA showed an effect of MDMA [$F_{(2,21)}$=6.93, p<0.01]. *Post hoc* comparisons revealed a dose-dependent increase in amplitude 3 hr following MDMA (20 mg/kg) administration only when compared to vehicle treated controls (p<0.01).

Somatosensory Cortex

MDMA provoked a dose-dependent decrease in MTT, CTT and increase in signal amplitude in somatosensory cortex 3 hr following drug administration when compared to vehicle treated controls (Table 3.3.2).

(A) MTT: ANOVA showed an effect of MDMA [$F_{(2,21)}$=7.733, p<0.01]. *Post hoc* comparisons revealed that MTT was decreased 3 hr following MDMA (5 and 20 mg/kg) administration when compared to vehicle treated controls (p<0.01). The magnitude of change in MTT was similar with both doses of MDMA.

(B) CTT: ANOVA showed an effect of MDMA [$F_{(2,20)}$=7.62, p<0.01]. *Post hoc* comparisons revealed that CTT was reduced 3 hr following MDMA (5 and 20 mg/kg) administration when compared to vehicle treated controls (p<0.05; p<0.01 respectively).

(C) Signal amplitude: ANOVA showed an effect of MDMA [$F_{(2,21)}$=10.29, p<0.001]. *Post hoc* comparisons revealed a dose-dependent increase in amplitude 3 hr following MDMA (20 mg/kg) administration only when compared to vehicle treated controls (p<0.01).

Other regional effects

Increases in signal amplitude ($p<0.05$) were observed in auditory cortex [$F_{(2,21)}=18.29$, $p<0.001$] (0.076 ± 0.004 and 0.095 ± 0.011) and thalamus [$F_{(2,21)}=6.77$, $p<0.01$] (0.084 ± 0.005 and 0.097 ± 0.011) following MDMA administration (20 mg/kg) and in the parietal association cortex [$F_{(2,20)}=14.12$, $p<0.001$] (0.076 ± 0.009 and 0.088 ± 0.007 and 0.076 ± 0.009 and 0.099 ± 0.012, respectively) following administration (5 and 20 mg/kg).

No changes in MTT, CTT or signal amplitude were observed in insular cortex, dorsal or ventral striatum, retrosplenial cortex, visual cortex or hippocampus at either of the doses assessed (data not shown).

Table 3.3.2 MDMA provokes a dose-dependent decrease in MTT and CTT with a corresponding increase in signal amplitude in cortex

		MTT (s)	*CTT (s)*	*Amplitude(a.u.)*
Secondary Motor	**Vehicle**	2.08 ± 0.09	1.72 ± 0.09	0.086 ± 0.003
	MDMA (5 mg/kg)	1.89 ± 0.07 **	1.6 ± 0.08	0.092 ± 0.003
	MDMA (20 mg/kg)	1.85 ± 0.04 **	1.41 ± 0.02 **	0.102 ± 0.003 **
Somatosensory	**Vehicle**	1.84 ± 0.06	1.56 ± 0.07	0.091 ± 0.003
	MDMA (5 mg/kg)	1.57 ± 0.04 **	1.35 ± 0.04 *	0.101 ± 0.004
	MDMA (20 mg/kg)	1.57 ± 0.07 **	1.29 ± 0.03 **	0.114 ± 0.004 **

Data are expressed as mean ± SEM (n=8). *p<0.05, **p<0.01 vs. vehicle (Dunnett's *post hoc* test).

3.3.3 Cortical and striatal 5-HT and 5-HIAA concentration following MDMA administration

In the time course experiment ANOVA of cortical 5-HT concentration showed an effect of MDMA [$F_{(1,28)}$=15.87, p<0.001]. *Post hoc* comparisons revealed a decrease in 5-HT concentration 3 and 24 hr following drug administration when compared to vehicle treated controls (Figure 3.3.3 (A)). ANOVA of 5-HIAA concentration also showed an effect of MDMA [$F_{(1,28)}$=11.29, p<0.01] and a time x MDMA interaction [$F_{(1,28)}$=4.25, p<0.05]. *Post hoc* comparisons revealed a decrease in cortical 5-HIAA concentration 3 but not 24 hr following drug administration when compared to vehicle treated controls (Figure 3.3.3 (B)). By contrast to the cortex, MDMA failed to influence 5-HT (Figure 3.3.3 (E)) concentration in the striatum.

ANOVA of striatal 5-HIAA (Figure 3.3.3(F)) concentration showed an effect of MDMA [$F_{(1,28)}$=17.83, p<0.001], time [$F_{(1,28)}$=40.8, p<0.001] and a time x MDMA interaction [$F_{(1,28)}$=6.57, p<0.05]. *Post hoc* comparisons revealed a reduction in striatal 5-HIAA concentration 3 but not 24 hr following drug administration when compared to vehicle treated controls.

Student's *t*-test revealed that cortical 5-HT concentration was significantly reduced (p<0.05) 1 hr following MDMA administration in comparison to vehicle treated controls (509.5 ± 37.44 and 334.7 ± 62.11, respectively). There was no significant change in 5-HIAA level 1 hr following MDMA administration. By contrast to the cortex, MDMA failed to influence 5-HT concentration in the striatum. Student's *t*-test revealed that 5-HIAA was

significantly reduced ($p<0.05$) 1 hr following MDMA administration in comparison to vehicle treated controls (813.8 ± 17.6 and 733.4 ± 27.55, respectively).

MDMA (5 and 20 mg/kg) produced a dose-dependent reduction in cortical 5-HT (Figure 3.3.3 (C)) and 5-HIAA (Figure 3.3.3 (D)) concentration 3 hr following drug administration. ANOVA of 5-HT and 5-HIAA concentration showed an effect of MDMA [$F_{(2,21)}=10.6$, $p<0.001$] and [$F_{(2,21)}=15.28$, $p<0.001$], respectively.

ANOVA of striatal 5-HIAA concentration showed an effect of MDMA [$F_{(2,21)}=11.04$, $p<0.001$]. Striatal 5-HIAA concentration (Figure 3.3.3 (H)) was reduced following MDMA (20 but not 5 mg/kg) when compared to vehicle treated controls. In a similar fashion to the time course experiment MDMA failed to influence striatal 5-HT concentration (Figure 3.3.3 (G)).

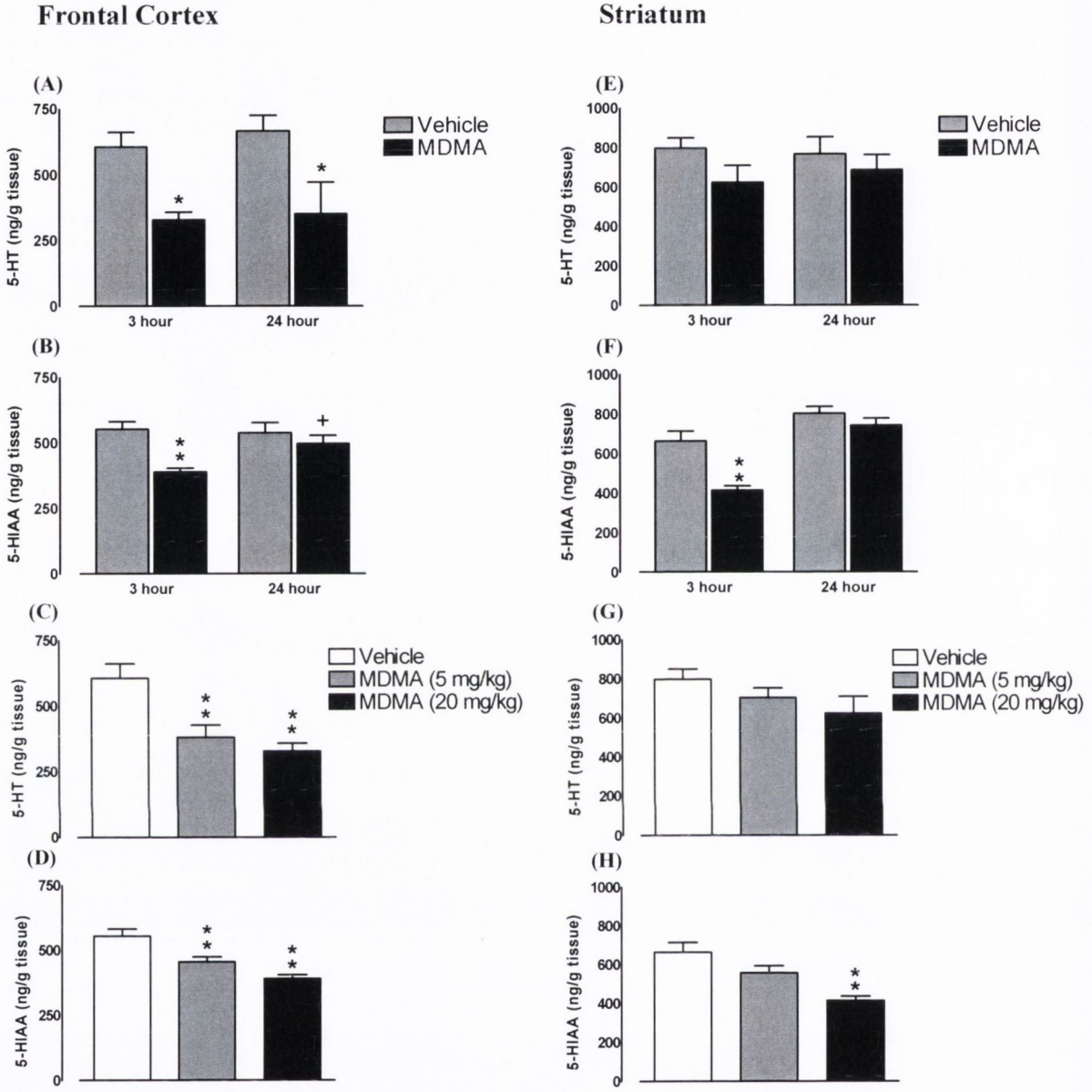


Figure 3.3.3 MDMA provokes a time dependent decrease in 5-HT and 5-HIAA concentration Animals received MDMA (5 or 20 mg/kg) and btASL was performed 3 or 24 hr after drug administration. Cortical and striatal 5-HT and 5-HIAA concentration were subsequently determined post-mortem. Panel 1 (A) and (B) shows the time course and (C) & (D) the dose related effects of MDMA in the frontal cortex. Panel 2 (E) and (F) shows the time course and (G) & (H) the dose related effects of MDMA in the striatum. Data are

expressed as mean ± SEM (n=8). * $p<0.05$; ** $p<0.01$ vs. vehicle at corresponding time point. + $p<0.05$ vs. MDMA (3 hr.) (Student Newman-Keuls or Dunnett's *post hoc* test).

3.4 Discussion

In the present investigation MDMA provoked a time- and dose-dependent decrease in MTT and CTT coupled with an increase in signal amplitude when compared to vehicle treated controls. As the transit times are inversely related to perfusion (MTT is inversely proportional to CBF while CTT is inversely proportional to CBF squared), reductions in MTT and CTT may be taken as evidence for increased CBF in response to MDMA. Related to this, increases in signal amplitude reflect an increase in CBV in line with increased perfusion (Kelly *et al.*, 2010). The effects of MTT, CTT and signal amplitude were regionally dependent with changes significant in frontal cortical regions, including the motor and somatosensory cortex, but not sub-cortical or posterior cortical regions including striatum, hippocampus, thalamus, auditory, visual, retrosplenial and parietal association cortex. MDMA-induced reductions in transit times were most notable after 1 and 3 hr with effects significantly less apparent after 24 hr. In tandem, changes to signal amplitude and CBV were observed 1 and 3 hr, but absent 24 hr, following drug administration. When taken together the results indicate that MDMA provokes acute dose related increases in CBF and CBV in frontal cortical regions that persist for several hours but are reduced or absent within 24 hr.

As MDMA administration to rats has been reported previously to provoke a disruption to BBB permeability (Bankson, 2005; Sharma and Ali, 2008), the time course of brain clearance of gadolinium following intravenous infusion was determined 3 and 24 hr following MDMA administration, subsequent to the btASL measurements. No differences in clearance between drug treated and vehicle treated controls were observed in the frontal

cortex confirming that there was no obvious loss of barrier integrity in this region (Figure 3.3.1.2 (A) - (C)). In a parallel set of animals, all groups were also checked for the extravasation of Evans blue dye into brain parenchyma following intravenous administration. No evidence of the presence of dye was found throughout the parenchyma confirming that MDMA did not influence barrier integrity (data not shown). Thus in the present investigation changes in CBF and CBV occurred independently of any observable loss of integrity to the BBB. Disruption to the BBB however is likely to occur with higher doses of MDMA than those used in the present investigation. MDMA (10 mg/kg; i.p.) administered to rats every two hours for a total of 4 injections was reported to result in a breakdown of the barrier evident through the extravasation of trypan blue dye in brain parenchyma (Bankson, 2005). Moreover Sharma and Ali (2008) reported increased extravasation of Evans blue dye into brain parenchyma following administration of a single high dose of MDMA (40 mg/kg) to rats in various brain regions including cerebral cortex, hippocampus, cerebellum, thalamus and hypothalamus 4 hr after drug administration.

As expected, in advance of MR scanning, MDMA provoked a dose- and time-dependent increase in body temperature when compared to vehicle treated controls that had returned to normal 24 hr following drug administration. Moreover MDMA was associated with a significant reduction in cortical 5-HT and 5-HIAA concentration 3 hr following drug administration. Whilst the reduction in 5-HT persists for 24 hr, cortical 5-HIAA concentration returns to control levels over this time indicating a recovery of 5-HT metabolism following MDMA administration. These effects of MDMA on cortical 5-HT and 5-HIAA were dose-dependent. By contrast to the cortex however, MDMA failed to produce a reduction in striatal 5-HT concentration although striatal 5-HIAA concentration was reduced 3 hr following drug administration. Overall the effects obtained are consistent

with numerous previous reports where the 5-HT depleting action of MDMA is most notable in cortical areas (Baumann *et al.*, 2007; Wang *et al.*, 2004). The sparing of 5-HT innervated sub-cortical areas such as the striatum suggests that 5-HT neurons innervating the cerebral cortex are more susceptible to MDMA. Unlike the majority of amphetamine studies (Rosa-Neto *et al.*, 2004, for review) we found no activation by MDMA of CBF in the basal ganglia which leads us to suggest that the net effects of MDMA on the release of 5-HT and/or dopamine in the rat striatum did not alter local afferent activity sufficiently to perturb CBF. Our findings are also consistent with Rosa-Neto (2004) who reported that MDMA failed to influence CBF in the pig striatum yet drug effects were apparent in the frontal cortex. Such observations in relation to the regional specificity of the effects of MDMA are also in line with the findings of some clinical investigations where PET and structural brain imaging have shown that cerebral SERT binding is affected in cortical regions in abstinent ecstasy users leading investigators to propose that behavioural problems during abstinence might be related to changes in blood perfusion limited to cortical regions (Kish *et al.*, 2010)

One important difference between experiments performed to date may relate to whether or not anesthesia was employed. btASL MRI is performed under anesthesia and when compared to other imaging approaches, behavioural changes are not able to be expressed. Thus the effects observed with btASL cannot arise due to feedback from drug-induced behaviours and this may in part account for the fact that observations with btASL are anatomically less widespread with the exception of the neocortex when compared to other investigations where anesthesia was not employed.

Taken in combination, the data from the time and dose response experiments presented suggest that MDMA-induced increases in CBF and CBV are more localised to frontal

cortical regions, a finding that is consistent with the results of Quate *et al.*, (2004) who reported 19% increases in CBF in frontal cortex using an autoradiographic technique 25 min after MDMA (15 mg/kg; i.p.) administration to rats. In addition to this however, in this same study, marked decreases in CBF were reported in many regions including primary sensory (superior colliculus, medial geniculate) and limbic areas (anterior thalamus, dorsal subiculum). There was no evidence that decreases in blood flow were organised according to the vascular territories of the principal cerebral arteries. Perfusion in brain regions supplied by the anterior (anterior cingluate) and middle cerebral arteries were either unchanged (somatosensory and piriform cortex) or increased (frontal cortex). The increase in cortical CBF occurred in the absence of any change in LCMRglu suggestive of a direct cerebrovascular response to MDMA independent of changes in metabolic demand. Changes in CBF are not always found to be directly related either quantitatively or qualitatively to changes underlying metabolic demand (Quate *et al.*, 2004). This uncoupling has been attributed to the fact that 5-HT possesses potent vasoactive properties and 5-HT fibres have been identified innervating cerebral arteries, arterioles and veins (Steinbusch, 1981). Thus there is the potential for 5-HT to play an important role in the regulation and modulation of haemodynamic processes independent of underlying metabolism, through vasoconstriction of cerebral blood vessels and consequent decreases in blood flow (Cohen *et al.*, 1996).

Whilst many of the effects of MDMA on neuronal function may be explained by the release of 5-HT, this action may not account for CBF and CBV changes observed in the present investigation. 5-HT is known to have predominant constrictive actions on blood vessels (Cohen *et al.*, 1996) which would reduce CBF, actions which are not consistent with the effects observed in this investigation. With MDMA-induced 5-HT release one would

anticipate reduced cerebrovascular perfusion. Some investigators have put this mechanism forward to account for decreased rCBF consistent with acute vasoconstriction associated with MDMA mediated serotonergic effects (Chang *et al.*, 2000; Reneman *et al.*, 2000). MDMA however, following an initial increase in 5-HT release, over a time course of hours results in an acute 5-HT depleted state. It is therefore not unreasonable to suggest that MDMA-induced 5-HT depletion with reduced perivascular 5-HT release and subsequent loss of 5-HT mediated constrictor tone may lead to vasodilatation and prevailing increased CBF.

Other monoamine neurotransmitters influenced by MDMA may also play a role in the effect observed. Dopamine generally mediates vasodilatation in cerebral vessels *in vitro* (Edvinsson *et al.*, 1985) which if transposed into the intact animal would result in increased blood flow. It has been proposed previously that MDMA may evoke dopamine release from perivascular nerves, and by inducing dilatation of the cerebral arteries, summate with the reduced constrictor tone that results from 5-HT depletion to reduce the upper limit of cerebrovascular autoregulation resulting in increases in CBF which are proportional to increases in systemic blood pressure. Such effects have been reported previously for amphetamine (Berntman *et al.*, 1978; Carlsson *et al.*, 1975; Florence *et al.*, 2000; Russo *et al.*, 1991). Acute 5-HT depletion coupled with increased extra-synaptic dopamine availability resulting from a single administration of MDMA may lead to loss of resting cerebrovascular constrictor tone and a possible focal loss of cerebrovascular autoregulatory capacity in areas known to be susceptible to vascular damage and stroke arising from hypertension. It is interesting to note that frontal cortex is the area of the brain where intracerebral haemorrhage has been reported clinically following exposure to MDMA (Harries & De Silva, 1992).

There is little evidence that central noradrenergic systems act upon resistance arterioles in the cerebrovascuar bed *in vivo* to alter cerebral blood flow, although the activation of sympathetic innervations of more proximal arteries does have a role to play in pressure autoregulation (Edvinsson *et al.,* 1977). Further studies will be required in order to elucidate the mechanisms underlying the effects of MDMA on cerebral perfusion.

Clinical implications of results and concluding remarks

In conclusion, this study provides important evidence regarding brain haemodynamic changes following acute administration of MDMA in a rodent model and suggests that MDMA produces cortical hyperperfusion possibly mediated by a decrease in cerebrovascular tone. Taken together with the ability of MDMA to produce sustained cardiovascular effects and hypertension (Gamma *et al.*, 2000; Ferrington *et al.*, 2006; Vollenweider *et al.*, 1998), such changes may have important implications in relation to increased risk of CVA and haemorrhagic stroke in recreational ecstasy users. The acute effects of MDMA on cerebral perfusion may go some way towards providing a mechanism to explain the occurrence of CVA in young people following ingestion of MDMA although it is important to note that few users of MDMA succumb to CVA. In this regard MDMA may contribute to pre-existing conditions or vulnerabilities such as congenital abnormalities in vascular structure or function. Nevertheless with a greater understanding of the effects of MDMA on cerebral perfusion, concerns have been raised that a proportion of those who use MDMA may suffer infarcts leading to cognitive decline stemming from a vascular rather than purely neuronal pathology (Ferrington *et al.*, 2006). Future clinical studies of MDMA users are likely to be directed towards correlating cognitive decline with small vessel disease and lacunar stroke as well as with loss of 5-HT nerve terminals.

Chapter 4

Investigation of the role of 5-HT and dopamine in mediating increased cortical perfusion following MDMA "Ecstasy"

Chapter 4: Investigation of the role of 5-HT and dopamine in mediating increased cortical perfusion following MDMA "Ecstasy"

4.1 Introduction

Previously we and others have reported that the recreational drug of abuse MDMA "Ecstasy" provokes regional, time and dose-dependent increases in cerebral perfusion and CBV (Reneman *et al.,* 2000; Rosa-Neto *et al.,* 2004; van Donkelaar *et al.,* 2010). This is a drug effect with potential adverse consequences, as MDMA use has been linked to the incidence of CVA, the mechanism of which warrants further investigation. Although numerous studies have been carried out on the effects of MDMA on CBF and CBV, the mechanism of action by which alterations to cerebral perfusion are mediated remains unclear.

It is widely reported that the pharmacological actions of MDMA result in the release of 5-HT and dopamine in several regions of the brain (Colado *et al.*, 2004; El-Mallakh and Abraham, 2007; Green *et al.*, 2003, for review; Gudelsky and Yamamoto, 2008; Shankaran and Gudelsky, 1998). A role for 5-HT in the regulation of cerebral perfusion has previously been described. Perivascular nerves which originate in the raphe nuclei contain 5-HT fibres which innervate cerebral microvasculature (Hamel, 2006, for review). Reduced blood flow in the cortex following raphe stimulation in rats can be abolished in the presence of 5-HT_2 receptor antagonists (Cao *et al.,* 1992). Activation of 5-HT_{1B} receptors located on cortical microvessels has both vasoconstrictor and in some cases vasodilatatory effects (Edvinsson *et al.,* 1987; Elhusseiny & Hamel, 2001) whereas the 5-HT_{1D} receptor agonist sumatriptan

reduces cortical blood flow (Kobari *et al.,* 1993). It has been reported that common carotid arterial blood flow is decreased following tonic release of 5-HT in cats (Gong *et al.,* 2002; Kuo *et al.,* 1999; Li *et al.,* 1996). Further studies also support a role for 5-HT_{2A} and 5-HT_{1B} receptors in mediating the vasoconstrictor response following cerebral ischemia (Bouchelet *et al.,* 2000; Hansen-Schwartz *et al.,* 2003; Nilsson *et al.,* 1999). Taken together reports to date implicate a predominant vasoconstrictive action of 5-HT on cerebral blood vessels.

While many of the effects of MDMA on neuronal function may be explained by the release of 5-HT, this action may not account for the vascular effects. 5-HT mediated vasoconstriction subsequent to MDMA induced neurotransmitter release would be consistent with a reduction, not an increase, in cerebral blood perfusion as has previously been reported. A role for 5-HT however may nevertheless be relevant on account of the ability of MDMA to promote central 5-HT depletion which may, in turn, relieve the constrictive actions of the transmitter, favouring vasodilatation. Dopamine on the other hand generally mediates vasodilatation in cerebral vessels *in vitro* (Edvinsson *et al.*, 1985), which if transposed into the intact animal would result in increased blood flow. Krimer and colleagues (1998) elucidated a role for dopaminergic transmission in cortical microcirculation. Dopaminergic neurons were shown to innervate blood vessels in the frontal lobe, particularly in the premotor and prefrontal cortex. In addition to this it was reported that dopamine produces vasomotor responses in the cortical vasculature *in vitro*. It has been reported that activation of dopamine D_1 and D_5 receptors are responsible for producing positive or increased CBF changes whereas stimulation of dopamine D_2 and D_3 receptors elicit negative or decreased CBF changes (Choi *et al.,* 2006). Further support for a role of dopamine in mediating increased cerebral perfusion in response to MDMA may be drawn from reports related to the effects of amphetamine administration. Chen and

colleagues (2005) observed increases in regional CBV (rCBV) following amphetamine administration (3 mg/kg; i.v.) that was potentiated with eticlopride (D_2/D_3 antagonist) and attenuated with quinpirole (D_2/D_3 agonist). In a subsequent report increased CBV in response to amphetamine or the DAT inhibitor, (-)2-β-carbomethoxy-3-β-(4-fluorophenyl)tropane (CFT), was attenuated by pre-treatment with the dopamine D_1 receptor antagonist SCH 23390 (Choi *et al.,* 2006). Amphetamine (1 mg/kg; i.v.) and the dopamine D_1/D_5 agonist dihydrexidine (3 mg/kg; i.v.) have subsequently been reported to increase rCBV whilst quinpirole (2 mg/kg; i.v.) provokes a reduction in rCBV (Chen *et al.,* 2010). Taken together, these findings suggest a potential role for dopamine in mediating increased cortical perfusion and CBV associated with MDMA-induced dopamine release. It is interesting to note however, that at lower doses of amphetamine (0.25 mg/kg) significant decreases in rCBV have been reported (Ren *et al.,* 2009). The authors suggested a switch in the balance of dopamine D_2/D_3 stimulation towards dopamine D_1/D_5 stimulation to account for the bi-directional dose related response to amphetamine.

Given the established role of 5-HT and dopamine in the regulation of cerebral perfusion, this study sets out to determine the mechanisms that mediate the ability of MDMA to increase cortical perfusion and volume in rats. First, we investigated if the effects of MDMA generalise to fenfluramine, a synthetic amphetamine that selectively induces the release of central 5-HT, or if the response to MDMA could be simulated by administration of the non-selective 5-HT_2 receptor agonist 2,5 dimethoxy-4-iodophenyl-aminopropane hydrochloride (DOI). Next, to assess if the effects of MDMA were dependent on endogenous 5-HT, the effects of 5-HT depletion on MDMA-induced changes in cortical perfusion were determined. In addition, inhibition of 5-HT transmission was assessed by prior administration of the non-selective 5-HT receptor antagonist metergoline. 5-HT

transporter availability is believed to be necessary for the uptake of MDMA or a metabolic by-product into 5-HT neurons in advance of provoking 5-HT release and consequent 5-HT depletion (Malberg *et al.*, 1996; McCann & Ricaurte, 2004; Piper *et al.*, 2008; Sanchez *et al.*, 2001; Schmidt, 1987). Pre-treatment with the selective 5-HT transporter inhibitor (SSRI) citalopram, the SSRI that is most selective for the 5-HT transporter and that has the least effect on cytochrome P450 (CYP) activity (Hemeryck & Belpaire, 2002), attenuates MDMA-induced 5-HT loss (Battaglia *et al.*, 1988; Piper *et al.*, 2008). The present experiment also tested the hypothesis that blocking the 5-HT transporter and resultant uptake of MDMA with citalopram will prevent the cortical perfusion changes associated with MDMA. Finally to elucidate a role for dopamine D_1 receptors, the effect of prior administration of the selective dopamine D_1 receptor antagonist SCH 23390 was assessed on MDMA-induced changes. The results show that 5-HT dependent and independent mechanisms unrelated to dopamine D_1 receptor activation are relevant to the mechanism underlying MDMA related increases in cortical perfusion.

4.2 Experimental Procedure

Study 1: Can the 5-HT releasing agent fenfluramine or the 5-HT$_2$ receptor agonist DOI mimic changes in cortical perfusion associated with MDMA?

Fenfluramine and MDMA-induced changes in MTT, CTT and signal amplitude were determined in parallel. Animals received a single administration of fenfluramine (10 mg/kg; i.p.), MDMA (20 mg/kg; i.p.) or vehicle (saline) and were placed into the MRI scanner 1 hr later. Perfusion and CBV were assessed 1 hr following drug administration as this time point corresponds to the peak hypothermic response observed following fenfluramine administration (Cryan *et al.*, 2000).

The effects of the non selective 5-HT$_2$ receptor agonist DOI (1 mg/kg; i.p.) were determined 1 and 3 hr following drug administration. DOI-induced head twitches and wet dog shakes, a behavioural response following activation of 5-HT$_2$ receptors, were monitored continuously until the animals were anaesthetised and placed into the MRI scanner.

Study 2: Can central 5-HT depletion or 5-HT receptor blockade influence MDMA-induced changes in cortical perfusion?

Central 5-HT depletion was induced as previously described (Vanattou-Saifoudine *et al.*, 2010a) by administration of the tryptophan hydroxylase inhibitor *para*-chlorophenylalanine (*p*CPA; 150mg/kg; i.p., once daily for 3 days). A 72 hr period was allowed to elapse following the last treatment with *p*CPA prior to challenge with MDMA (20mg/kg; i.p.) or vehicle (saline). Animals were subsequently placed into the MRI scanner 3 hr later.

The non-selective 5-HT receptor antagonist metergoline was used at a dose effective in blocking the *in vivo* effects induced by 5-HT receptor agonists in rats (Golozoubova *et al.*, 2006; Mokler *et al.*, 1983; Stachowicz *et al.*, 2007). Animals received either metergoline (4mg/kg; i.p.) or vehicle (0.5% Tween saline) and 30 min later were challenged with either MDMA (20 mg/kg; i.p.) or saline. Animals were subsequently placed in the MRI scanner 3 hr later.

Study 3: Can blockade of the 5-HT transporter prevent MDMA-induced changes in cortical perfusion?

Animals received either citalopram (30 mg/kg; i.p.) or vehicle (saline) and 30 min later were challenged with either MDMA (20 mg/kg; i.p.) or vehicle (saline). Animals were subsequently placed in the MRI scanner 3 hr later.

Study 4: Can prior treatment with the selective dopamine $D_{1/5}$ receptor antagonist, SCH 23390, influence MDMA-induced changes in cortical perfusion?

The selective dopamine $D_{1/5}$ receptor antagonist, SCH 23390, was used in this study at a dose of 1 mg/kg which blocks MDMA-induced hyperthermia (Vanattou-Saifoudine *et al.*, 2010a). Animals received either SCH 23390 (1 mg/kg; i.p.) or vehicle (saline) and 30 min later were challenged with either MDMA (20 mg/kg; i.p.) or vehicle (saline). Animals were subsequently placed into the MRI scanner 3 hr later.

4.3 Results

4.3.1 Fenfluramine, but not DOI, mimics MDMA-induced changes in cortical perfusion

Fenfluramine provoked a reduction in core body temperature. ANOVA showed an effect of fenfluramine [$F_{(2,42)}$=25.28, p<0.001] and a fenfluramine x time interaction [$F_{(4,42)}$=23.35, p<0.001]. *Post hoc* comparisons revealed a significant decrease in body temperature (p<0.001) 30 and 60 min following fenfluramine administration with a maximum decrease of 1.7 °C ± 0.44 °C when compared to an increase in vehicle treated controls (0.29 °C ± 0.25 °C). By contrast MDMA provoked an increase in core body temperature [$F_{(2,42)}$=25.28, p<0.0001] and an MDMA x time interaction [$F_{(4,42)}$=23.35, p<0.0001]. *Post hoc* comparisons revealed a significant increase in body temperature (p<0.001) 30 and 60 min following MDMA administration with a maximum body temperature increase of 1.78 °C ± 0.19 °C in comparison to an increase in vehicle treated controls (0.29 °C ± 0.25 °C). Both fenfluramine and MDMA provoked a decrease in MTT and CTT and a corresponding increase in signal amplitude in primary, secondary motor and somatosensory cortex. The magnitude of change in each region was similar with both drugs.

Primary Motor Cortex

ANOVA of MTT and CTT showed a drug effect [$F_{(2,21)}$=21.44, p<0.001] and [$F_{(2,21)}$=14.15, p<0.001], respectively. *Post hoc* comparisons revealed that MTT and CTT were decreased 1 hr following fenfluramine and MDMA administration when compared to vehicle treated controls (Figure 4.3.1.1 (A) and (B)).

ANOVA of signal amplitude showed a drug effect [$F_{(2,21)}$=11.93, p<0.001]. *Post hoc* comparisons revealed that signal amplitude was increased 1 hr following fenfluramine and MDMA administration when compared to vehicle treated controls (Figure 4.3.1.1 (C)).

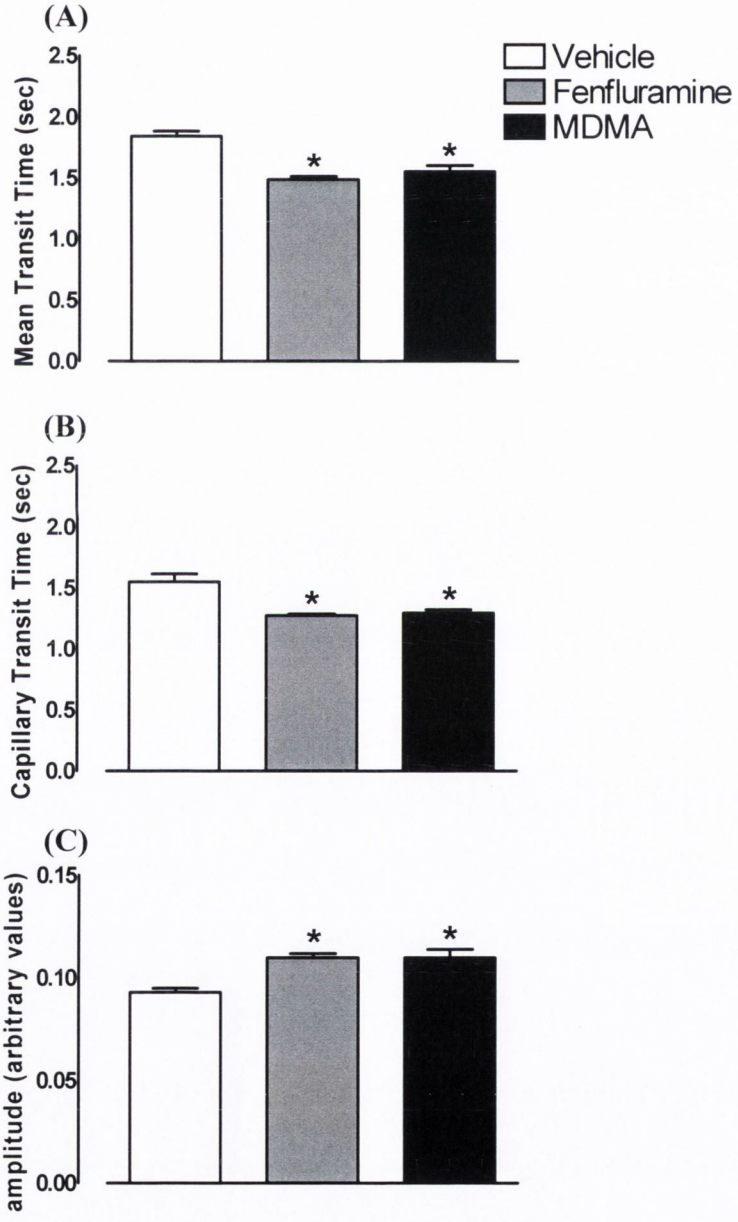


Figure 4.3.1.1 Fenfluramine, like MDMA, provokes a decrease in MTT and CTT with a corresponding increase in signal amplitude in the primary motor cortex

Fenfluramine (10 mg/kg) and MDMA (20 mg/kg) provoke a decrease in (A) MTT and (B) CTT and an increase in (C) signal amplitude 1 hr following drug administration when compared to vehicle treated controls in the primary motor cortex. Data are expressed as mean ± SEM (n=8). *$p<0.01$ vs. vehicle (Dunnett's *post hoc* test).

Secondary Motor Cortex

ANOVA of MTT and CTT showed a drug effect [$F_{(2,21)}$=17.22, p<0.001] and [$F_{(2,21)}$=11.23, p<0.001], respectively. *Post hoc* comparisons revealed that MTT and CTT were decreased 1 hr following fenfluramine and MDMA administration when compared to vehicle treated controls.

ANOVA of signal amplitude showed a drug effect [$F_{(2,21)}$=9.32, p<0.01]. *Post hoc* comparisons revealed that signal amplitude was increased 1 hr following fenfluramine and MDMA administration when compared to vehicle treated controls (Table 4.3.1).

Somatosensory Cortex

ANOVA of MTT and CTT showed a drug effect [$F_{(2,21)}$=20.71, p<0.001] and [$F_{(2,21)}$=13.77, p<0.001], respectively. *Post hoc* comparisons revealed that MTT and CTT were decreased 1 hr following fenfluramine and MDMA administration.

ANOVA of signal amplitude showed a drug effect [$F_{(2,21)}$=12.91, p<0.001]. *Post hoc* comparisons revealed that signal amplitude was increased 1 hr following fenfluramine and MDMA administration when compared to vehicle treated controls (Table 4.3.1).

Other regional effects

ANOVA of signal amplitude showed an effect of drug in the auditory cortex [$F_{(2,21)}$=3.79, p<0.05]. *Post hoc* comparisons revealed that fenfluramine (0.098 ± 0.004) and MDMA (0.089 ± 0.005) increased signal amplitude when compared to vehicle treated controls (0.08 ± 0.003; p<0.05).

ANOVA of signal amplitude showed an effect of drug in the thalamus [$F_{(2,21)}$=5.79, p<0.01]. *Post hoc* comparisons revealed that fenfluramine (0.1 ± 0.003) and MDMA (0.097

± 0.003) increased signal amplitude when compared to vehicle treated controls (0.08 ± 0.003; $p<0.05$). There were no changes in MTT or CTT in either of these regions.

In addition there were no changes in MTT, CTT or signal amplitude observed in dorsal or ventral striatum, insular, visual, parietal association or retrosplenial cortex or hippocampus (data not shown).

Table 4.3.1 Fenfluramine and MDMA provoke a decrease in MTT and CTT with a corresponding increase in signal amplitude in cortex

		MTT (s)	*CTT (s)*	*Amplitude(a.u.)*
Secondary Motor	**Vehicle**	1.98 ± 0.06	1.64 ± 0.08	0.09 ± 0.003
	Fenfluramine	1.55 ± 0.02 *	1.28 ± 0.007 *	0.107 ± 0.002 *
	MDMA	1.64 ± 0.07 *	1.34 ± 0.05 *	0.105 ± 0.004 *
Somatosensory	**Vehicle**	1.71 ± 0.04	1.51 ± 0.07	0.097 ± 0.003
	Fenfluramine	1.42 ± 0.02 *	1.23 ± 0.009 *	0.117 ± 0.002 *
	MDMA	1.44 ± 0.04 *	1.25 ± 0.02 *	0.119 ± 0.004 *

Data are expressed as mean ± SEM (n=8). * $p<0.01$ vs. vehicle (Dunnett's *post hoc* test).

Cortical 5-HT determination

ANOVA of cortical 5-HT concentration showed a drug effect [$F_{(2,20)}$=4.24, p<0.05]. *Post hoc* comparisons revealed that 5-HT concentration was reduced 1 hr following administration of both drugs when compared to vehicle treated controls (Figure 4.3.1.2 (A)).

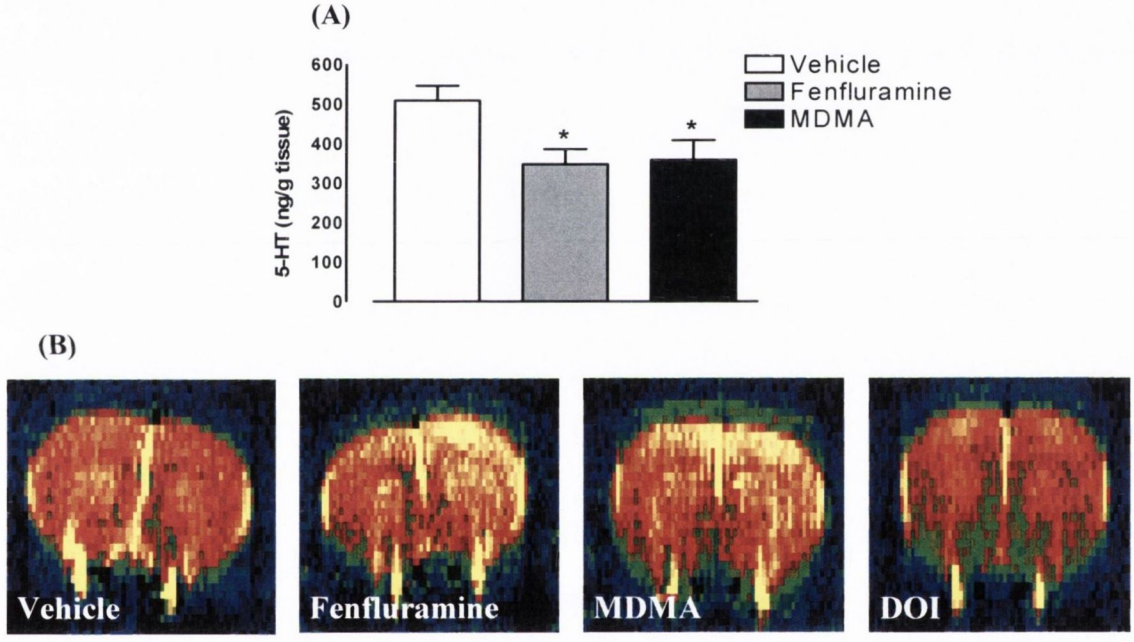


Figure 4.3.1.2 Cortical 5-HT concentration following fenfluramine administration and associated representative blood volume maps

Fenfluramine (10 mg/kg) and MDMA (20 mg/kg) decreased cortical 5-HT concentration (A) 1 hour following drug administration in comparison to vehicle treated controls. CBV maps depicting fenfluramine- and MDMA-induced increases in CBV 1 hr following drug administration in a representative coronal brain slice (B). DOI did not mimic these changes. Data are expressed as mean ± SEM (n=8). ** p<0.01 vs. vehicle (Dunnett's *post hoc* test).

The effect of direct 5-HT_2 receptor activation was also assessed by administration of the 5-HT_2 receptor agonist DOI. DOI (1 mg/kg) induced head twitches and wet dog shakes in rats 1 and 3 hr following administration consistent with the activation of central 5-$HT_{2A/2C}$ receptors as previously described (Kohnomi *et al.,* 2008). Student's *t*-test revealed an increase in head twitches ($p<0.001$) 1 hr (45 ± 12) and 3 hr (44 ± 7) following DOI administration in comparison to vehicle treated controls (0 ± 0 and 0.1 ± 0.1), respectively. An increase in the number of wet dog shakes ($p<0.001$) 1 hr (38 ± 9) and 3 hr (41 ± 8) following DOI administration was observed in comparison to vehicle treated controls (0.5 ± 0.3 and 0.6 ± 0.3), respectively. DOI did not provoke a change in MTT, CTT or signal amplitude in any of the brain regions tested (data not shown).

4.3.2 5-HT depletion provokes an increase in cortical perfusion and potentiates MDMA related changes

Primary Motor Cortex

ANOVA of MTT and CTT showed an effect of *p*CPA [$F_{(1,26)}$=28.33, p<0.0001] and [$F_{(1,26)}$=10.38, p<0.001] and an effect of MDMA [$F_{(1,26)}$=34.23, p<0.0001] and [$F_{(1,26)}$=22.26, p<0.001], respectively. *Post hoc* comparisons revealed that MTT and CTT were decreased following *p*CPA and MDMA treatments alone when compared to vehicle treated controls (p<0.01) and that *p*CPA potentiated the effects of MDMA when compared to MDMA treatment alone (p<0.01) (Figure 4.3.2.1 (A) and (B)).

ANOVA of signal amplitude showed an effect of *p*CPA [$F_{(1,26)}$=15.59, p<0.001] and MDMA [$F_{(1,26)}$=27.63, p<0.0001]. *Post hoc* comparisons revealed that signal amplitude was increased following *p*CPA and MDMA treatments alone when compared to vehicle treated controls (p<0.01) and that *p*CPA potentiated the effects of MDMA when compared to MDMA treatment alone (p<0.01) (Figure 4.3.2.1 (C)).

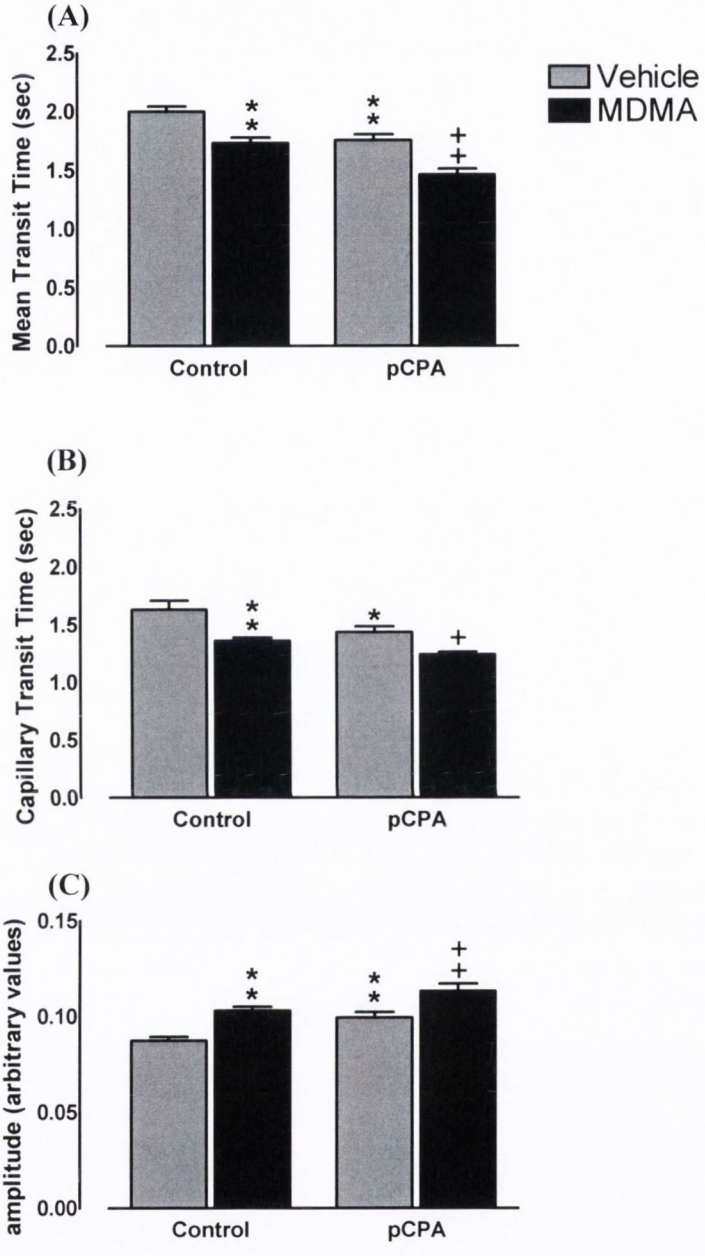


Figure 4.3.2.1 5-HT depletion provokes a decrease in MTT and CTT and increase in signal amplitude and potentiates the response to MDMA in the primary motor cortex

MDMA (20 mg/kg) provokes a decrease in (A) MTT and (B) CTT and an increase in (C) signal amplitude following drug administration when compared to vehicle treated controls in the primary motor cortex. *p*CPA treatment (150 mg/kg; i.p., daily for 3 days followed by

72 hr recovery) potentiates the MDMA-induced changes in (A) MTT, (B) CTT and (C) signal amplitude. Data are expressed as mean ± SEM (n=6-8). * $p<0.05$; ** $p<0.01$ vs. vehicle. + $p<0.05$; ++ $p<0.01$ vs. *p*CPA + vehicle. (Student Newman-Keuls *post hoc* test).

Secondary Motor Cortex

ANOVA of MTT and CTT showed an effect of *p*CPA [$F_{(1,26)}=14.64$, $p<0.001$] and [$F_{(1,26)}=4.99$, $p<0.05$] and an effect of MDMA [$F_{(1,26)}=9.78$, $p<0.01$] and [$F_{(1,26)}=7.88$, $p<0.01$], respectively. *Post hoc* comparisons revealed that MTT and CTT were decreased following *p*CPA and MDMA treatments alone when compared to vehicle treated controls ($p<0.05$) and that *p*CPA potentiated the effects of MDMA when compared to MDMA treatment alone ($p<0.01$).

ANOVA of signal amplitude showed an effect of *p*CPA [$F_{(1,26)}=5.11$, $p<0.05$] and MDMA [$F_{(1,26)}=7.2$, $p<0.01$]. *Post hoc* comparisons revealed that signal amplitude was increased following MDMA when compared to vehicle treated controls ($p<0.05$) and that *p*CPA potentiated the effects of MDMA when compared to MDMA treatment alone ($p<0.01$) (Table 4.3.2).

Somatosensory Cortex

ANOVA of MTT and CTT showed an effect of *p*CPA [$F_{(1,26)}$=17.74, p<0.001] and [$F_{(1,26)}$=12.11, p<0.01] and an effect of MDMA [$F_{(1,26)}$=20.8, p<0.01] and [$F_{(1,26)}$=18.94, p<0.001], respectively. *Post hoc* comparisons revealed that MTT and CTT were decreased following *p*CPA and MDMA treatments alone when compared to vehicle treated controls (p<0.01) and that *p*CPA potentiated the effects of MDMA when compared to MDMA treatment alone (p<0.01).

ANOVA of signal amplitude revealed an effect of *p*CPA [$F_{(1,26)}$=10.36, p<0.01] and MDMA [$F_{(1,26)}$=32.5, p<0.0001]. *Post hoc* comparisons revealed that signal amplitude was increased following *p*CPA and MDMA treatments alone when compared to vehicle treated controls (p<0.05) and that *p*CPA potentiated the effects of MDMA when compared to MDMA treatment alone (p<0.01) (Table 4.3.2).

Other regional effects

ANOVA of signal amplitude in the auditory cortex showed an effect of *p*CPA [$F_{(1,26)}$=19.68, p<0.01] and MDMA [$F_{(1,26)}$=30.32, p<0.0001]. *Post hoc* comparisons revealed that signal amplitude was increased following *p*CPA (0.108 ± 0.003) and MDMA (0.095 ± 0.004) when compared to vehicle treated controls (0.092 ± 0.003 and 0.076 ± 0.001 respectively) in the absence of a change in MTT or CTT.

ANOVA of signal amplitude in the parietal association cortex revealed an effect of *p*CPA [$F_{(1,26)}$=7.42, p<0.05] and an effect of MDMA [$F_{(1,26)}$=18.05, p<0.001]. *Post hoc* comparisons revealed that signal amplitude was increased following *p*CPA (0.105 ± 0.005) and MDMA (0.099 ± 0.004) when compared to vehicle treated controls (0.092 ± 0.005 and 0.073 ± 0.003 respectively) in the absence of a change in MTT or CTT.

ANOVA of signal amplitude in the thalamus showed an effect of *p*CPA [$F_{(1,26)}$=7.05, p<0.05] and MDMA [$F_{(1,26)}$=5.33, p<0.05]. *Post hoc* comparisons revealed that signal amplitude was increased following *p*CPA (0.105 ± 0.005) and MDMA (0.096 ± 0.004) when compared to vehicle treated controls (0.09 ± 0.006 and 0.084 ± 0.002) in the absence of a change in MTT or CTT.

No changes in MTT, CTT or signal amplitude were observed in dorsal or ventral striatum, insular or retrosplenial cortex or hippocampus (data not shown).

Table 4.3.2 pCPA potentiates MDMA-induced decreases in MTT and CTT and corresponding increase in signal amplitude in cortex

		Control			*p*CPA		
		MTT (s)	*CTT (s)*	*Amplitude(a.u.)*	*MTT (s)*	*CTT (s)*	*Amplitude(a.u.)*
Secondary	**Vehicle**	2.08 ± 0.06	1.72 ± 0.09	0.087 ± 0.003	1.85 ± 0.06 *	1.53 ± 0.07 *	0.096 ± 0.004 *
	MDMA	1.89 ± 0.06 *	1.49 ± 0.07 *	0.099 ± 0.002 *	1.65 ± 0.05 +	1.34 ± 0.04	0.105 ± 0.003
Somatosensory	**Vehicle**	1.85 ± 0.06	1.56 ± 0.07	0.09 ± 0.003	1.59 ± 0.06 *	1.34 ± 0.05 *	0.104 ± 0.005 **
	MDMA	1.57 ± 0.06 **	1.3 ± 0.03 **	0.114 ± 0.004 **	1.34 ± 0.03 ++	1.19 ± 0.014 ++	0.127 ± 0.004 +

Data are expressed as mean ± SEM (n=6-8). * $p<0.05$; ** $p<0.01$ vs. vehicle control. + $p<0.05$; ++ $p<0.01$ vs. *p*CPA vehicle (Student Newman-Keuls *post hoc* test).

Cortical 5-HT determination

ANOVA of cortical 5-HT concentration revealed an effect of *p*CPA [$F_{(1,26)}$=44.91, p<0.0001], an effect of MDMA [$F_{(1,26)}$=11.77, p<0.01] and a *p*CPA x MDMA interaction [$F_{(1,26)}$=9.1, p<0.01]. *Post hoc* comparisons revealed that 5-HT concentration was significantly reduced following *p*CPA (p<0.01) or MDMA (p<0.05) treatments in comparison to vehicle treated controls.

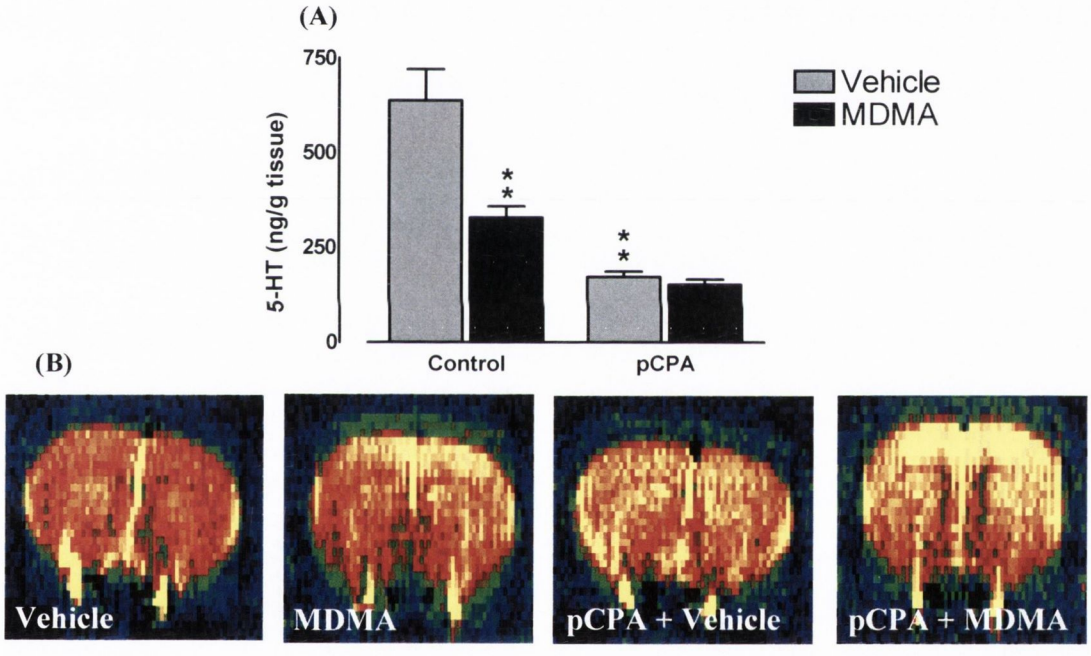


Figure 4.3.2.2 Cortical 5-HT concentration following pCPA treatment and associated representative blood volume maps

MDMA administration (20 mg/kg) and *p*CPA treatment (150 mg/kg; i.p., once daily for 3 days followed by 72 hr recovery) reduce cortical 5-HT concentration (A) in comparison to vehicle treated controls. CBV maps depicting MDMA-induced increases in CBV that are

potentiated following *p*CPA treatment (B). Data are expressed as mean ± SEM (n=6-8). ** $p<0.01$ vs. vehicle control animals (Student Newman-Keuls *post hoc* test).

The effect of blocking 5-HT receptors was assessed by pre-treatment with the non-selective 5-HT receptor antagonist metergoline. Metergoline failed to provoke a change in MTT, CTT or signal amplitude in any of the brain regions tested or to attenuate MDMA-induced decreases in MTT and CTT and increase in signal amplitude in primary, secondary motor and somatosensory cortex (data not shown).

4.3.3 MDMA-induced changes in cortical perfusion are not mediated by 5-HT depletion

Primary Motor Cortex

ANOVA of MTT and CTT showed an effect of MDMA [$F_{(1,22)}$=21.52, p<0.001] and [$F_{(1,22)}$=13.11, p<0.01], respectively. *Post hoc* comparisons revealed that MTT and CTT were decreased 3 hr following MDMA and this effect was not influenced by pre-treatment with citalopram.

ANOVA of signal amplitude showed an effect of MDMA [$F_{(1,22)}$=16.41, p<0.001]. *Post hoc* comparisons revealed that signal amplitude was increased 3 hr following MDMA administration and that this effect was not influenced by pre-treatment with citalopram (Table 4.3.4).

Secondary Motor Cortex

ANOVA of MTT and CTT showed an effect of MDMA [$F_{(1,22)}$=17.29, p<0.001] and [$F_{(1,22)}$=7.99, p<0.01], respectively. *Post hoc* comparisons revealed that MTT and CTT were decreased 3 hr following MDMA administration and this effect was not influenced by pre-treatment with citalopram.

ANOVA of signal amplitude showed an effect of MDMA [$F_{(1,22)}$=10.19, p<0.01]. *Post hoc* comparisons revealed that signal amplitude was increased 3 hr following MDMA administration and that this effect was not influenced by pre-treatment with citalopram (Table 4.3.4).

Somatosensory Cortex

ANOVA of MTT and CTT showed an effect of MDMA [$F_{(1,22)}$=20.88, p<0.001] and [$F_{(1,22)}$=11.16, p<0.01], respectively. *Post hoc* comparisons revealed that MTT and CTT were decreased 3 hr following MDMA administration and this effect was not influenced by pre-treatment with citalopram.

ANOVA of signal amplitude showed an effect of MDMA [$F_{(1,22)}$=14.96, p<0.001]. *Post hoc* comparisons revealed that signal amplitude was increased 3 hr following MDMA administration and that this effect was not influenced by pre-treatment with citalopram (Table 4.3.4).

Cortical 5-HT determination

ANOVA of cortical 5-HT concentration showed an effect of citalopram [$F_{(1,22)}$=16.42, p<0.001] and a citalopram x MDMA interaction [$F_{(1,22)}$=39.13, p<0.001]. *Post hoc* comparisons revealed a decrease in 5-HT concentration 3 hr following MDMA administration when compared to vehicle treated controls. Pre-treatment with citalopram completely blocked the MDMA related decrease in cortical 5-HT concentration (Figure 4.3.3 (A)).

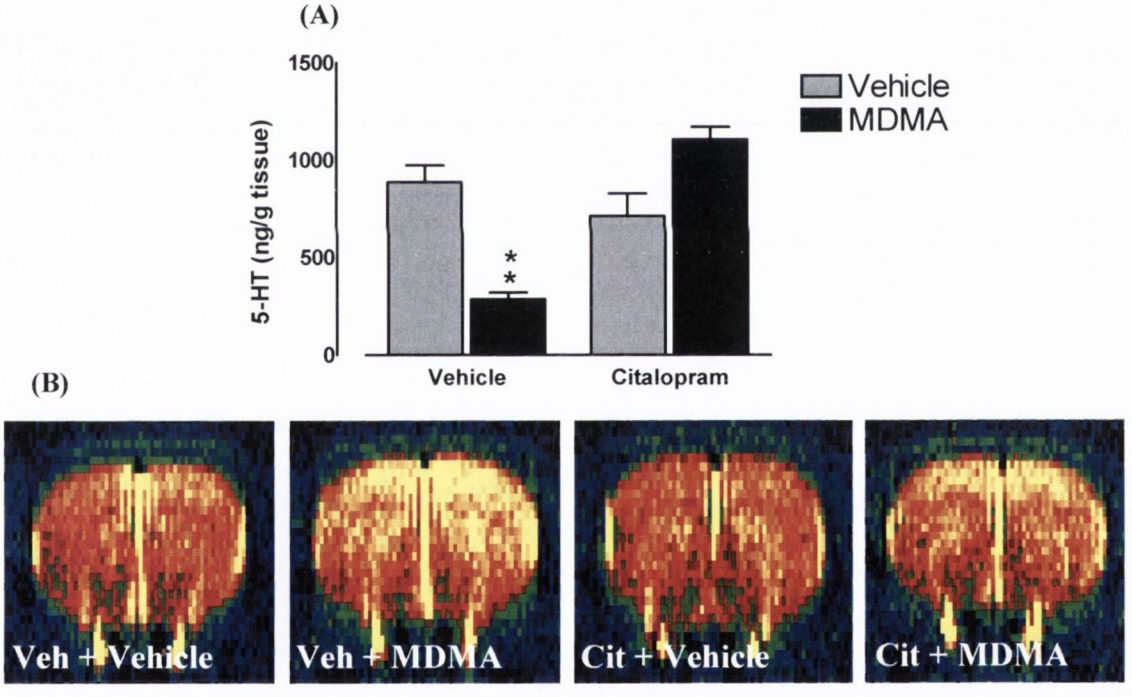


Figure 4.3.3 Cortical 5-HT concentration following citalopram pre-treatment and associated representative blood volume maps

MDMA-induced decreases in cortical 5-HT concentration are attenuated following citalopram pre-treatment (A). CBV maps depicting MDMA-induced increases in CBV are

uninfluenced by citalopram pre-treatment (B). Data are expressed as mean ± SEM (n=6-7).

** $p<0.01$ vs. vehicle treated controls (Student Newman-Keuls *post hoc* test).

4.3.4 Prior administration of SCH 23390 provokes a decrease in perfusion of the visual and parietal association cortex but fails to influence MDMA related changes in cortical perfusion

Pre-treatment with SCH 23390 alone had no effect on core body temperature when compared to vehicle treated controls. MDMA provoked an increase in body temperature (maximum increase 2.01°C ± 0.16°C) which was attenuated by prior treatment with SCH 23390 (-0.45°C ± 0.17°C).

SCH 23390 treatment alone provoked an increase in MTT and CTT and a decrease in signal amplitude in visual and parietal association cortex 3.5 hr following administration in comparison to vehicle treated controls. ANOVA of MTT and CTT showed an interaction between SCH 23390 and MDMA in the visual [$F_{(1,26)}$=8.26, p<0.01]; [$F_{(1,26)}$=8.68, p<0.01] and parietal association [$F_{(1,26)}$=6.57, p<0.05]; [$F_{(1,26)}$=5.78, p<0.05] cortex, respectively. ANOVA of signal amplitude showed an interaction between SCH 23390 and MDMA in the visual [$F_{(1,26)}$=5.84, p<0.05] and parietal association [$F_{(1,26)}$=5.38, p<0.05] cortex. P*ost hoc* comparisons revealed an increase in MTT and CTT and a decrease in signal amplitude 3.5 hr following SCH 23390 in comparison to vehicle treated controls. ANOVA of signal amplitude in the parietal association cortex showed an effect of MDMA [$F_{(1,26)}$=11.46, p<0.01]. *Post hoc* comparisons revealed that signal amplitude was increased following MDMA (0.09 ± 0.005) when compared to vehicle treated controls (0.076 ± 0.003) in the absence of a change in MTT or CTT. No perfusion related changes were observed in the visual cortex following MDMA administration alone.

Primary Motor Cortex

ANOVA of MTT and CTT showed an effect of MDMA [$F_{(1,28)}$=35.26, p<0.0001] and [$F_{(1,28)}$=19.36, p<0.001], respectively. *Post hoc* comparisons revealed that MTT and CTT were decreased 3 hr following MDMA administration. Pre-treatment with SCH 23390 did not influence this response.

ANOVA of signal amplitude showed an effect of MDMA [$F_{(1,28)}$=25.48, p<0.0001]. *Post hoc* comparisons revealed that signal amplitude was increased 3 hr following MDMA administration. Pre-treatment with SCH 23390 did not influence this response (Table 4.3.4).

Secondary Motor Cortex

ANOVA of MTT and CTT showed an effect of MDMA [$F_{(1,28)}$=22.12, p<0.0001] and [$F_{(1,28)}$=12.44, p<0.01], respectively. *Post hoc* comparisons revealed that MTT and CTT were decreased 3 hr following MDMA administration in comparison to vehicle treated controls. Pre-treatment with SCH 23390 did not influence this response.

ANOVA of signal amplitude showed an effect of MDMA [$F_{(1,28)}$=15.15, p<0.001]. *Post hoc* comparisons revealed that signal amplitude was increased 3 hr following MDMA administration in comparison to vehicle treated controls. Pre-treatment with SCH 23390 failed to attenuate this response (Table 4.3.4).

Somatosensory Cortex

ANOVA of MTT and CTT showed an effect of MDMA [$F_{(1,28)}$=46.12, p<0.0001] and [$F_{(1,28)}$=21.38, p<0.0001], respectively. *Post hoc* comparisons revealed that MTT and CTT were decreased 3 hr following MDMA administration in comparison to vehicle treated controls. Pre-treatment with SCH 23390 failed to attenuate this response.

ANOVA of signal amplitude showed an effect of MDMA [$F_{(1,28)}$=35.21, p<0.0001]. *Post hoc* comparisons revealed that signal amplitude was increased 3 hr following MDMA administration in comparison to vehicle treated controls. Pre-treatment with SCH 23390 failed to attenuate this response (Table 4.3.4).

Other regional effects

Pre-treatment with SCH 23390 failed to attenuate the MDMA-induced increase in signal amplitude in the parietal association cortex (data not shown).

No changes in MTT, CTT or signal amplitude were observed in dorsal or ventral striatum, insular, auditory or retrosplenial cortex, thalamus or hippocampus (data not shown).

Table 4.3.4 Pre-treatment with citalopram or SCH 23390 fails to attenuate the MDMA-induced decreases in MTT and CTT and corresponding increase in signal amplitude in primary, secondary motor and somatosensory cortex

		Vehicle			Citalopram		
		MTT (s)	*CTT (s)*	*Amplitude(a.u.)*	*MTT (s)*	*CTT (s)*	*Amplitude(a.u.)*
Primary	**Vehicle**	1.79 ± 0.05	1.47 ± 0.07	0.095 ± 0.004	1.82 ± 0.05	1.48 ± 0.06	0.092 ± 0.001
	MDMA	1.56 ± 0.06 *	1.30 ± 0.04 *	0.109 ± 0.004 *	1.56 ± 0.05 **	1.28 ± 0.02 *	0.105 ± 0.003 *
Secondary	**Vehicle**	1.85 ± 0.05	1.49 ± 0.05	0.09 ± 0.004	2.02 ± 0.07	1.71 ± 0.11	0.092 ± 0.004
	MDMA	1.56 ± 0.09 *	1.33 ± 0.07 *	0.111 ± 0.005 *	1.69 ± 0.07 *	1.43 ± 0.06	0.103 ± 0.004 *
Somatosensory	**Vehicle**	1.65 ± 0.03	1.47 ± 0.05	0.098 ± 0.003	1.71 ± 0.06	1.49 ± 0.07	0.097 ± 0.003
	MDMA	1.41 ± 0.03 **	1.25 ± 0.03 *	0.116 ± 0.006 *	1.51 ± 0.07 *	1.33 ± 0.06 *	0.112 ± 0.004 *
		Vehicle			**SCH 23390**		
		MTT (s)	*CTT (s)*	*Amplitude(a.u.)*	*MTT (s)*	*CTT (s)*	*Amplitude(a.u.)*
Primary	**Vehicle**	1.81 ± 0.05	1.52 ± 0.08	0.093 ± 0.004	1.85 ± 0.04	1.60 ± 0.05	0.089 ± 0.002
	MDMA	1.61 ± 0.07 **	1.36 ± 0.07 *	0.107 ± 0.004 *	1.46 ± 0.03 **	1.24 ± 0.01 *	0.111 ± 0.004 **
Secondary	**Vehicle**	1.86 ± 0.05	1.53 ± 0.06	0.090 ± 0.004	1.94 ± 0.06	1.62 ± 0.09	0.090 ± 0.004
	MDMA	1.61 ± 0.09 *	1.38 ± 0.07	0.108 ± 0.005 *	1.58 ± 0.05 **	1.28 ± 0.05 *	0.108 ± 0.005 *
Somatosensory	**Vehicle**	1.63 ± 0.03	1.47 ± 0.04	0.097 ± 0.003	1.61 ± 0.03	1.47 ± 0.03	0.095 ± 0.002
	MDMA	1.44 ± 0.04 **	1.27 ± 0.03 **	0.116 ± 0.005 **	1.40 ± 0.03 **	1.30 ± 0.04 **	0.117 ± 0.004 **

Data are expressed as mean ± SEM (n=6-8). * $p<0.05$; ** $p<0.01$ vs. corresponding vehicle (Student Newman-Keuls *post hoc* test).

4.4 Discussion

4.4.1 Increased cortical perfusion following MDMA is mimicked by 5-HT depletion

In line with previous observations MDMA provoked a decrease in MTT and CTT with a corresponding increase in signal amplitude in the primary, secondary motor and somatosensory cortex indicating an increase in perfusion and CBV. Since MDMA is a well known releaser of central 5-HT, it was conceivable that MDMA-induced 5-HT release may underlie the effects observed. Administration of the 5-HT releasing agent fenfluramine similarly resulted in decreases in MTT and CTT with a corresponding increase in signal amplitude in cortical areas. Increased cortical perfusion and CBV following administration of two 5-HT releasing agents, lend further support for a role of 5-HT in mediating the increase in cerebral perfusion and blood volume observed. As fenfluramine and MDMA produced opposing actions on core body temperature, with fenfluramine provoking a hypothermic response and MDMA provoking a hyperthermic response, increased cortical perfusion and blood volume are likely to occur independently from drug-induced changes in core body temperature.

By contrast to the indirect 5-HT agonists MDMA and fenfluramine, the direct 5-HT_2 receptor agonist DOI failed to mimic the increase in cortical perfusion and CBV despite the appearance of DOI-induced 5-HT_2 mediated behaviours including head twitches and wet dog shakes as previously described (Kohnomi *et al.,* 2008). The results suggest that the increase in cortical perfusion and blood volume obtained with MDMA or fenfluramine is independent of 5-HT_2 receptor activation. Further support for the non-participation of 5-HT

receptors is drawn from the lack of response to administration of the non selective 5-HT receptor antagonist metergoline and lack of interaction between metergoline and MDMA.

In an attempt to elucidate a role for 5-HT loss in mediating MDMA-induced changes in cortical perfusion and blood volume, the response to MDMA was determined in 5-HT depleted animals. *p*CPA-induced 5-HT depletion provoked an MDMA-like response alone with enhanced effects when combined with MDMA administration. As MDMA, fenfluramine and *p*CPA produce a depletion in cortical 5-HT concentration, it is not unreasonable to suggest that a loss of the vasoconstrictive action of 5-HT on cerebral microvessels, relief from which may in turn promote vasodilatation, may underlie an increase in cortical perfusion and blood volume associated with these agents.

4.4.2 Increased cortical perfusion following MDMA is not mediated by 5-HT depletion

In order to further elucidate a role for 5-HT depletion in mediating the increase in cerebral perfusion following MDMA administration, animals were pre-treated with citalopram to block the uptake of MDMA into 5-HT neurons. Pre-treatment with citalopram failed to attenuate the MDMA-induced decrease in MTT and CTT and the increase in signal amplitude despite a complete attenuation of MDMA-induced cortical 5-HT loss. Thus 5-HT depletion alone, although provoking an MDMA-like response, does not provide a mechanism that accounts for MDMA related changes in cortical perfusion and blood volume. Furthermore the fact that MDMA, fenfluramine or *p*CPA-induced 5-HT depletion produced effects alone, which were not evident following treatment with metergoline, suggests that the effects of 5-HT depletion are independent of a corresponding reduction in 5-HT receptor activation and are likely to depend instead on a factor which works

independently of, but in concert with, 5-HT depletion to effect an increase in cortical perfusion.

4.4.3 A role for dopamine?

MDMA has direct agonist actions at 5-HT receptors which may account for its ability to provoke toxicity in the absence of endogenous 5-HT. Such actions include the ability of 5-HT receptors to influence dopamine release as 5-HT_2 receptors play an important role in the regulation of central dopaminergic function (Doly *et al.,* 2008; Di Matteo *et al.,* 2008, for review; Gudelsky & Yamamoto, 2008). Enhanced release of dopamine in the central nervous system following MDMA administration has been widely reported (Bankson & Cunningham, 2001; Colado *et al.,* 2004; Green *et al.,* 2003; Shankaran & Gudelsky, 1998). Previous studies have implicated a role for dopamine and dopamine D_1 receptors in mediating CBF changes in response to amphetamine administration as described earlier (Chen *et al.,* 2005; Chen *et al.,* 2010; Choi *et al.,* 2006; Krimer *et al.,* 1998). In the current investigation, SCH 23390 treatment alone produced increases in MTT and CTT with a corresponding decrease in signal amplitude in both the visual and the parietal association cortex. These decreases in CBF and CBV following administration of a dopamine receptor antagonist suggest a role for dopamine D_1/D_5 receptors in regulating cerebrovascular tone in these regions and are in accordance with previous reports implicating a role for dopamine in mediating CBF and CBV changes to amphetamine administration (Choi *et al.,* 2006). In the current investigation however pre-treatment with SCH 23390 did not attenuate MDMA-induced perfusion changes.

4.4.4 A role for direct vascular actions of MDMA?

Previous studies have reported a direct action on α-adrenoceptors in mediating blood pressure and heart rate changes following MDMA administration and in particular $\alpha_{2A/D}$-adrenoceptors in mediating a vasoconstrictive response (Bexis & Docherty, 2006; Vandeputte & Docherty, 2002). There is however little evidence that central noradrenergic systems act upon resistance arterioles in the cerebrovascuar bed *in vivo* to alter cerebral blood flow (Edvinsson *et al.,* 1977). An assessment of a role for adrenergic receptors in contributing to the MDMA-induced changes in CBF and CBV may nevertheless need to be addressed. MDMA produces sustained cardiovascular effects and hypertension (Gamma *et al.,* 2000; Ferrington *et al.,* 2006; Vollenweider *et al.,* 1998). When taken together with the potential for MDMA to evoke dopamine release from perivascular nerves and resultant dilatation of cerebral arteries in addition to the reduced constrictor tone that may result from 5-HT depletion, cerebrovascular autoregulation may be impaired resulting in increases in CBF proportional to increases in systemic blood pressure. Such mechanisms have been proposed and described previously to account for changes in cerebral perfusion associated with amphetamine (Berntman *et al.,* 1978; Carlsson *et al.,* 1975; Florence *et al.,* 2000; Russo *et al.,* 1991).

Despite the lack of evidence in support of a role for dopamine D_1 receptors, it is still possible that dopamine may play a role in mediating the cerebral perfusion changes associated with MDMA in a receptor-independent fashion. Vaarman and co-workers (2010) have shown that SCH 23390 pre-treatment did not block Ca^{2+} signalling in astrocytes. As astrocytes are an important component of the neurovascular unit (Carmignoto & Gómez-Gonzalo, 2010), it is possible that dopamine may exert an influence independently of receptor activation. While SCH 23390 has been reported to block the CBF effects of

amphetamine (Choi *et al.,* 2006), there are alternative mechanisms which are influenced by dopamine to effect CBF changes. A future direction could be to investigate this by blocking enzymes in the cyclooxygenase pathway which are utilised by astrocytes to enable them to enact their functions on vasculature (Koehler, 2009, for review).

In addition to the potential receptor-independent mechanism of MDMA-induced increases in cortical perfusion, metabolites of MDMA may play a role in mediating the cerebral perfusion changes observed. It has been widely reported that the serotonergic neurotoxicity associated with MDMA use depends on the systemic administration (Molliver *et al.,* 1986; Paris & Cunningham, 1992; Schmidt & Taylor, 1988; Schmidt *et al.,* 1987) and metabolism of the drug (de la Torre *et al.,* 2004; Farré, 2004). 3,4-dihydroxymethamphetamine (*N*-Me-α-MeDA) and its associated conjugates have been reported to be associated with MDMA-induced neurotoxicity (Bai *et al.,* 1999; 2001; Jones *et al.,* 2004). Although reports to date have not investigated the role that these MDMA metabolites may play in mediating CBF and CBV changes associated with MDMA, a future study may investigate whether direct injection of MDMA into the brain can reproduce the increases in cortical perfusion observed following systemic administration of the drug.

4.4.5 Concluding remarks

Our investigations into the role of 5-HT and dopamine D_1 receptors in mediating MDMA-induced increases in cortical blood perfusion and volume leads us to suggest a role for 5-HT depletion in mediating this action. 5-HT depletion may act to reduce vasoconstrictive tone which in turn contributes to increases in perfusion. However 5-HT depletion alone is unlikely to be the sole contributing factor as blockade of MDMA-induced 5-HT depletion fails to reverse the changes in cortical perfusion associated with MDMA administration. A

role for dopamine D_1 receptor-mediated vasodilatation is unlikely on account of the lack of interaction with SCH 23390. Mechanisms independent of 5-HT such as direct drug/metabolite action, or 5-HT and dopamine D_1 receptor-independent regulation of the cerebral microvasculature unit should also be considered.

Chapter 5

Investigation of the long-term effects of repeated MDMA "Ectsasy" exposure on cerebral cortical perfusion with btASL MRI in rats

Chapter 5: Investigation of the long-term effects of repeated MDMA "Ectsasy" exposure on cerebral cortical perfusion with btASL MRI in rats

5.1 Introduction

There is a growing body of literature reporting the neurotoxic long-term effects of exposure to MDMA in laboratory animals raising concerns over the safety of its recreational use. MDMA administration leads to the long-term depletion of central 5-HT and 5-HIAA in rats (Colado *et al.,* 1993; O'Shea *et al.,* 1998; Shankaran & Gudelsky, 1998; Thompson *et al.,* 2004; Wallace *et al.,* 2001). In a seminal report in non-human primates, Hatzidimitriou and colleagues (1999) reported 83-95% reductions in 5-HT immunoreactive axon density 2 weeks following MDMA (5 mg/kg; twice daily over 4 days) administration and a persistent reduction in the axon density 7 years later. Reduced [^{3}H] paroxetine binding to the 5-HT transporter, a hallmark of 5-HT nerve terminal integrity, has also been widely reported following MDMA administration. In Sprague-Dawley rats, cortical and striatal [^{3}H] paroxetine binding were reduced for up to 32 weeks and binding in the hippocampus was reduced for up to 1 year following MDMA exposure (10 mg/kg; i.p., four times daily) (Scanzello *et al.,* 1993). Cortical [^{3}H] paroxetine binding, in Dark Agouti rats, was reduced by 27% (Colado *et al.,* 1995) and by greater than 50% (O'Shea *et al.,* 1998) 7 days following MDMA exposure (10 and 15 mg/kg, respectively).

In support of such MDMA related toxicity being a cause for concern in humans, PET studies in humans, using [^{11}C]McN-5652, have reported reduced SERT binding in posterior cingulate gyrus, left caudate, thalamus, occipital visual cortex, medial temporal lobes and brainstem of current MDMA users (Buchert *et al.,* 2004) and global decreases in brain SERT in abstinent MDMA users (McCann *et al.,* 1998). Decreases in SERT binding positively correlated with the extent of previous MDMA use (McCann *et al.,* 1998). PET studies in abstinent MDMA users, using [^{11}C]DASB, reported reduced SERT binding in comparison to non-drug using controls (McCann *et al.,* 2005). SPECT with the SERT ligand [^{123}I]β-CIT has also been employed to determine the extent of change to 5-HT transporter density associated with MDMA use. Reneman and colleagues (2001) reported a significant decrease in [^{123}I]β-CIT binding in female, but not male, heavy MDMA users in comparison to controls leading them to suggest a possible sex difference in susceptibility to MDMA-induced serotonergic neurotoxicity. Semple and colleagues (1999) reported a reduction in ligand binding to SERT, particularly in the primary sensory-motor cortex, in male, regular ecstasy users in comparison to control subjects. Recent MDMA users were reported to have reduced mean cortical (encompassing an average of frontal, parietal and occipital cortex) binding of the 5-HT$_2$ receptor ligand [^{123}I]R91150 in comparison to former MDMA users and control subjects (Reneman *et al.,* 2000). The authors proposed MDMA-related 5-HT release as a likely contributing factor in the down-regulation of 5-HT$_2$ receptors and further suggested a potential risk of cerebrovascular events in MDMA users consequent to alterations in serotonergic neural transmission.

With accumulating evidence of long term 5-HT loss associated with exposure to MDMA it is important to consider long term functional consequences. As a role for 5-HT in the regulation of CBF has been described, it is of interest to assess if MDMA exposure and

subsequent long term 5-HT loss may lead to a change in cerebral perfusion. In this regard there are a number of reports to date which have attempted to address this question. Chang and colleagues (2000), using both ^{133}Xe and ^{99m}TcHMPAO SPECT and MRI, assessed CBF and CBV in abstinent MDMA users. Abstinent MDMA users showed no changes in rCBF in comparison to matched control subjects however, subjects who received MDMA and were scanned 2-3 weeks later showed decreases in global CBF and rCBF in many regions including middle and superior temporal cortex, globus pallidus, putamen, inferior, middle and superior parietal cortex and midbrain. In addition, it was found that these decreases in rCBF were more pronounced in subjects who received higher doses of MDMA. In contrast, an increase in global CBF was observed in the two subjects who received MDMA and were not scanned until more than 2 months after drug administration. ^{99m}TcHMPAO SPECT was carried out on a patient suffering from ecstasy intoxication 20 days following MDMA ingestion (Finsterer *et al.,* 2003). Reduced CBF was reported predominantly in the temporal and parietal regions however, 29 days later the SPECT had returned to normal. Both of these studies attributed prolonged vasoconstriction of the cerebral vessels, an adverse effect of MDMA-induced depletion of serotonin (5-HT) and its metabolites, to the decrease in rCBF following MDMA ingestion.

Reneman and colleagues (2000), in a study using both [^{123}I]R91150 SPECT, to assess for 5-HT$_2$ receptor density, and MRI, to assess rCBV values, reported ex-MDMA users to have higher rCBV values in globus pallidus and right thalamus in comparison to recent MDMA users and control subjects. These increases in rCBV following abstinence from MDMA were correlated with high [^{123}I]R91150 binding and it was suggested that

5-HT loss following MDMA administration leads to low synaptic 5-HT concentration and a consequent up-regulation of 5-HT_2 receptors.

In a subsequent study Reneman *et al.,* (2001) assessed frontal and occipital cortical and lentiform nucleus (consisting of the putamen and globus pallidus) rCBV in a group of MDMA users abstinent at least 3 weeks from MDMA and other drugs. rCBV was increased in the globus pallidus of MDMA users. Increased rCBV was speculated to be a result of loss of tonic serotonergic vasoconstrictive effects of globus pallidal arterial vessels with resulting relative vasodilatation producing increased blood vessel volume. In the same study Reneman and colleagues also conducted diffusion weighted imaging to assess for differences in the apparent diffusion coefficient (ADC) of water. This technique can be used to detect changes in the free movement of water and can indirectly assay loss of structural integrity for example with regard to fibre bundles (Parker, 2004, for review). In this analysis the globus pallidus of MDMA users was also the only brain region affected, with increased ADC in the MDMA cohort. Reneman and colleagues (2001) speculated that the altered ADC values might be attributed to a loss of serotonergic axons within the globus pallidus.

These studies indicate that MDMA exposure is associated with both decreases and increases in rCBF and rCBV. In addition to these opposing effects on perfusion however, there are limitations associated with these experiments. Firstly, the retrospective nature of the studies fails to address the fact that there may be pre-existing differences in CBF and CBV between drug users and non-users. In addition to this, MDMA users may use additional recreational drugs and although subjects are required to abstain from MDMA use prior to the study, the potential remains that any changes

observed may be, in some way, attributable to the actions of other drugs in use. In order to further understand the effects of MDMA, animal studies that investigate CBF and CBV changes following MDMA administration are required.

It is clear from animal studies that under different conditions, MDMA administration has been reported to cause both decreases (Ferrington *et al.,* 2006; Quate *et al.,* 2004) and increases (Rosa-Neto *et al.,* 2004) in LCBF as described earlier. Studies of the long-term effects of MDMA on CBF have also previously been carried out in rodent models. Ferrington and colleagues (2006) using [^{14}C] iodoantipyrine autoradiography investigated LCBF changes 3 weeks following MDMA (15 mg/kg) administration and reported no differences between MDMA treated and vehicle treated animals. Animals that were administered MDMA on the day of testing showed decreases in LCBF in 12 of the brain regions analysed including habenula, cingulate cortex, thalamus, nucleus accumbens, hypothalamus and superior colliculus in comparison to vehicle treated animals. No LCBF changes were reported in animals pre-treated with MDMA and administered saline on the experimental day, 3 weeks later. Ferrington suggested a role for an MDMA-induced reduction in cerebrovascular constrictor tone in mediating the long-term changes associated with MDMA administration. Increases in LCMRglu were also reported in 15 of the 44 brain areas investigated in this group of animals. It was proposed that such an uncoupling of LCBF from underlying metabolic demand may provide the basis for oligaemia-induced pathological changes in the brain.

van Donkelaar and colleagues (2010) repeatedly administered MDMA (20 mg/kg; i.p., twice daily over 4 days) to Wistar rats and 3 weeks later reported increases in CBF in prefrontal cortex, lateral amygdala, substantia nigra and locus coeruleus in comparison to vehicle treated controls. These findings suggest that the administration regime of

MDMA, used in particular investigations, may produce opposing long-term CBF changes. McBean and colleagues also showed that 6-9 weeks after treatment with MDA, a demethylated form of MDMA and also a 5-HT specific neurotoxin, rats showed focal increases in CBF in excess of metabolic demand (McBean *et al.,* 1990).

We have previously reported with the MR perfusion technique btASL, that acute administration of the recreational drug MDMA "ecstasy" to rats promotes a dose related increase in cerebral cortical perfusion determined by a reduction in the transit time of labelled arterial water and an increase in signal amplitude indicative of increased cortical CBV. The effects were restricted to defined areas of the cerebral cortex including primary and secondary motor and somatosensory cortex and occurred in a time and dose related manner. In a follow up series of experiments to address the mechanism underlying MDMA related effects on cerebral perfusion, and as MDMA provokes a depletion in cortical 5-HT concentration, it was determined that 5-HT depletion mimics, but does not mediate, the MDMA related increase in cortical perfusion. As MDMA is a recreational drug that is taken repeatedly by recreational users over short periods and MDMA provokes a long-term reduction in central 5-HT concentration, it was also of interest to determine if repeated exposure to MDMA with subsequent long-term central 5-HT loss may provoke sustained alterations in cerebral cortical perfusion and CBV in the rodent model established. Moreover it was of interest to determine if prior exposure to MDMA and subsequent long-term cortical 5-HT loss would influence the response to acute challenge with MDMA. The results failed to show that repeated MDMA administration and 5-HT loss could produce a sustained effect on cerebral cortical perfusion. The response to acute MDMA challenge however was attenuated suggesting

that a functional adaptation occurs in response to prior exposure. The implications of these results are discussed.

5.2 Experimental Procedure

MDMA (5 or 10 mg/kg; i.p.) or saline (0.89%) was administered four times per day over 2 consecutive days. Core body temperature measurements were taken prior to each injection to assess for any MDMA-induced changes. Animals remained in their home cages for a further 8 weeks. On the day of testing animals received a single administration of MDMA (20 mg/kg; i.p.) or vehicle (saline). Animals were anaesthetised and placed into the MRI scanner 3 hr later.

Long-term cortical 5-HT loss was not observed in the animals treated with MDMA (5 mg/kg) (438 ± 10 ng/g tissue) in comparison to vehicle treated control animals (427 ± 24 ng/g tissue). As previously reported acute MDMA challenge provoked a reduction in MTT and CTT and an increase in signal amplitude in the primary, secondary motor and somatosensory cortex when compared to vehicle treated controls. In addition, acute MDMA challenge provoked a decrease ($p<0.05$) in MTT and CTT with a corresponding increase ($p<0.05$) in signal amplitude in animals who received the repeated regime of MDMA 8 weeks prior to challenge (1.57 ± 0.007, 1.28 ± 0.004 and 0.109 ± 0.003, respectively) in comparison to saline treated controls (1.96 ± 0.13, 1.64 ± 0.12 and 0.09 ± 0.005, respectively) in primary motor cortex. Similar effects of acute MDMA challenge were observed in secondary motor and somatosensory cortex in animals exposed to repeated MDMA. The results indicated that there was no change in the acute response to MDMA challenge following prior exposure to low dose MDMA. This outcome prompted a second experiment using a higher dose of MDMA (10 mg/kg) in the repeated regime to effect a long-term loss in cortical 5-HT concentration.

5.3 Results

5.3.1 Prior exposure to MDMA has no effect alone but attenuates increased cerebral cortical perfusion induced by acute MDMA challenge

Repeated MDMA (10 mg/kg; i.p., four times daily over 2 days) administration provoked a hyperthermic response (1.35 ± 0.22°C, n=17) on the first day of treatment in comparison to vehicle treated controls (-0.475 ± 0.23°C, n=17). Repeated MDMA administration on the second day of treatment increased body temperature (0.45 ± 0.13°C, n=17), but not to the same extent as treatment on the first day, in comparison to vehicle treated controls (-0.288 ± 0.11°C, n=17).

As previously reported MDMA induced a reduction in MTT and CTT and an increase in signal amplitude in motor and somatosensory cortex in comparison to vehicle treated controls. Prior exposure to MDMA (10, but not 5, mg/kg, four times daily for 2 days followed by 8 weeks) had no effect alone but attenuated the acute response to MDMA-induced perfusion changes. Prior exposure to MDMA (10, but not 5, mg/kg) provoked a long-term (8 week) reduction in cortical 5-HT concentration.

Primary Motor Cortex

(A) MTT: ANOVA showed an effect of acute MDMA challenge [$F_{(1,30)}=7$, $p<0.05$] and an MDMA pre-treatment x acute MDMA challenge interaction [$F_{(1,30)}=8.57$, $p<0.01$]. *Post hoc* comparisons revealed that acute MDMA challenge produced a

decrease in MTT 3 hr following administration in comparison to vehicle treated control animals. Prior MDMA did not influence MTT when compared to controls. In addition acute MDMA challenge failed to provoke a decrease in MTT in the prior MDMA exposed group.

(B) CTT: ANOVA showed an effect of MDMA pre-treatment [$F_{(1,30)}$=5.24, p<0.05]. *Post hoc* comparisons revealed that acute MDMA challenge produced a decrease in CTT 3 hr following administration in comparison to vehicle treated control animals. Prior MDMA did not influence CTT when compared to controls. In addition acute MDMA challenge failed to provoke a decrease in CTT in the prior MDMA exposed group.

(C) Signal amplitude: ANOVA did not show an effect of acute MDMA challenge [$F_{(1,30)}$=3.27, p=0.08], albeit approaching significance. *Post hoc* comparisons revealed that acute MDMA challenge produced an elevated signal amplitude in comparison to vehicle treated controls, however statistical significance was not achieved (p=0.08) (Figure 5.3.1 (A)).

Secondary Motor Cortex

(A) MTT: ANOVA showed an effect of acute MDMA challenge [$F_{(1,30)}$=6.98, p<0.05] and an MDMA pre-treatment x acute MDMA challenge interaction [$F_{(1,30)}$=4.17, p=0.05]. *Post hoc* comparisons revealed that acute MDMA challenge produced a decrease in MTT 3 hr following administration in comparison to vehicle treated control animals. Prior MDMA did not influence MTT when compared to controls. In addition acute MDMA challenge failed to provoke a decrease in MTT in the prior MDMA exposed group.

(B) CTT: ANOVA showed an effect of acute MDMA challenge [$F_{(1,30)}$=14.47, p<0.001]. *Post hoc* comparisons revealed that acute MDMA challenge produced a decrease in CTT 3 hr following administration in comparison to vehicle treated control animals. Prior MDMA did not influence CTT when compared to controls. In addition acute MDMA challenge failed to provoke a decrease in CTT in the prior MDMA exposed group.

(C) Amplitude: ANOVA did not show an MDMA pre-treatment x acute MDMA challenge interaction [$F_{(1,30)}$=3.16, p=0.08], albeit approaching significance. *Post hoc* comparisons revealed that acute MDMA challenge produced an elevated signal amplitude in comparison to vehicle treated controls, however statistical significance was not achieved (p=0.08) (Figure 5.3.1 (B).

Somatosensory Cortex

(A) MTT: ANOVA showed an effect of acute MDMA challenge [$F_{(1,30)}$=4.17, p=0.05]. *Post hoc* comparisons revealed that acute MDMA challenge produced a decrease in MTT 3 hr following administration in comparison to vehicle treated control animals. Prior MDMA did not influence MTT when compared to controls. In addition acute MDMA failed to provoke a decrease in MTT in the prior MDMA exposed group.

(B) CTT: ANOVA showed an effect of acute MDMA challenge [$F_{(1,30)}$=6.82, p<0.05]. *Post hoc* comparisons revealed that MDMA challenge produced a decrease in CTT 3 hr following administration in comparison to vehicle treated control animals. Prior MDMA did not influence CTT when compared to controls. In addition acute MDMA challenge failed to provoke a decrease in CTT in the prior MDMA exposed group.

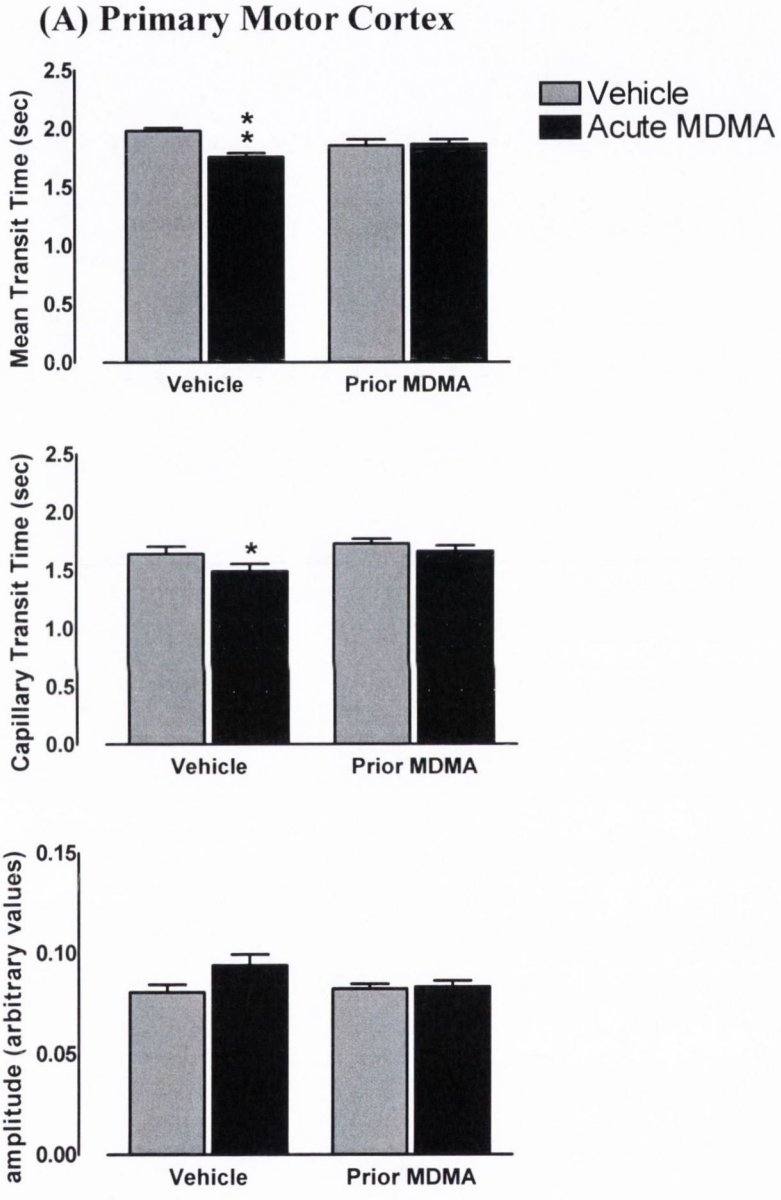
(C) Amplitude: ANOVA showed an effect of acute MDMA challenge [$F_{(1,30)}$=9.01, p<0.01] and an MDMA pre-treatment x acute MDMA challenge interaction [$F_{(1,30)}$=4.47, p<0.05]. *Post hoc* comparisons revealed that acute MDMA challenge produced an increase in signal amplitude 3 hr following administration in comparison to vehicle treated control animals. Prior MDMA did not influence amplitude when compared to controls. In addition acute MDMA challenge failed to provoke an increase in signal amplitude in the prior MDMA exposed group (Figure 5.3.1 (C).

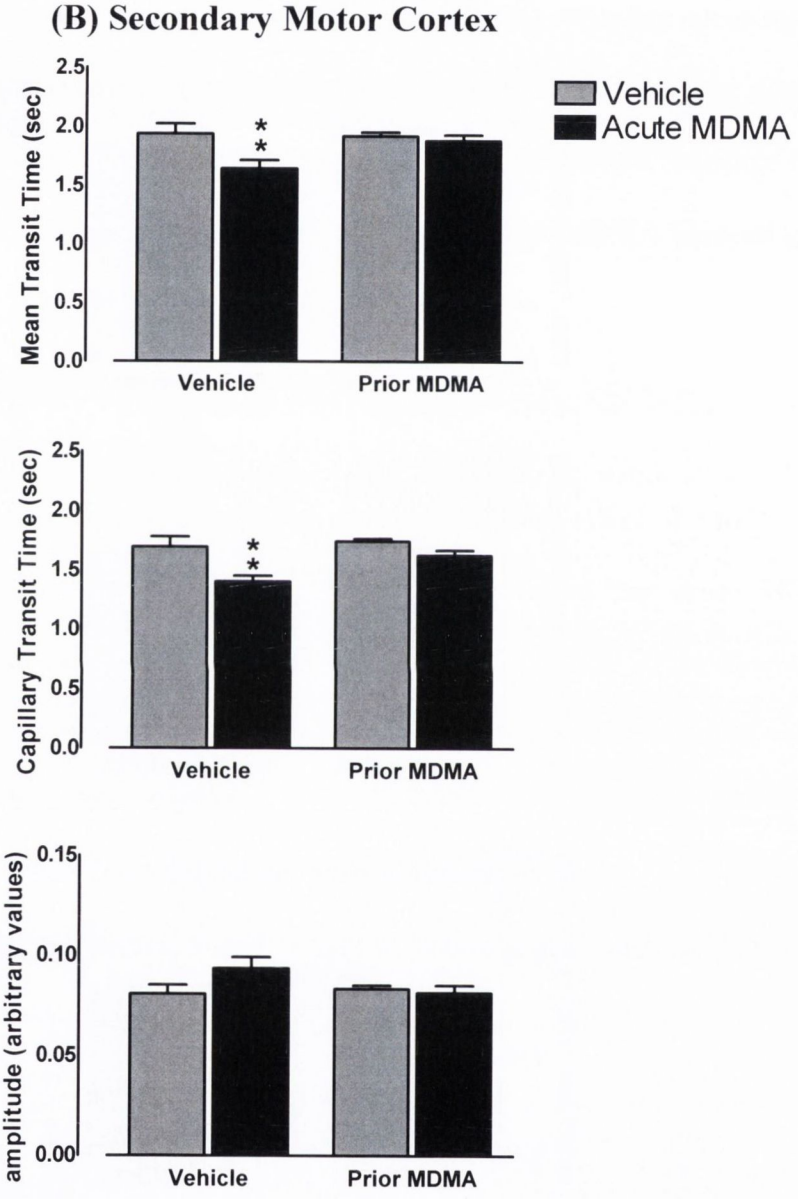
Other regional effects

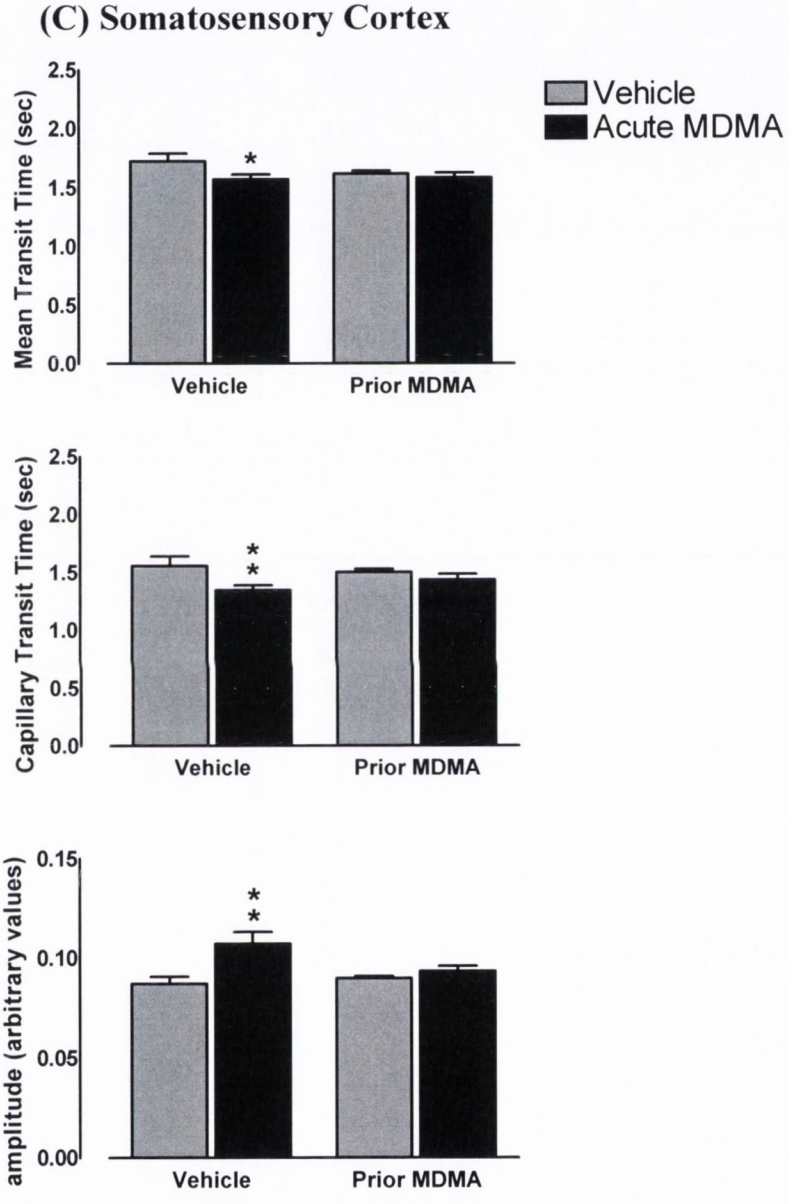
ANOVA of signal amplitude in the auditory cortex showed an effect of MDMA pre-treatment [$F_{(1,30)}$=8.03, p<0.01]. *Post hoc* comparisons revealed that acute MDMA challenge produced an increase in signal amplitude (0.088 ± 0.003), in the absence of any change in MTT or CTT, 3 hr following administration in comparison to vehicle treated control animals (0.078 ± 0.003).

No changes in MTT, CTT or amplitude were observed in insular, visual, parietal association or retrosplenial cortex, dorsal or ventral striatum, hippocampus or thalamus (data not shown).

Figure 5.3.1 Prior exposure to MDMA "ecstasy" attenuates increased cortical perfusion associated with acute MDMA challenge in rats




(B) Secondary Motor Cortex
Vehicle
Acute MDMA
Mean Transit Time (sec)
2.5
2.0
1.5
1.0
0.5
0.0
**
Vehicle
Prior MDMA
Capillary Transit Time (sec)
2.5
2.0
1.5
1.0
0.5
0.0
**
Vehicle
Prior MDMA
amplitude (arbitrary values)
0.15
0.10
0.05
0.00
Vehicle
Prior MDMA


(C) Somatosensory Cortex
Vehicle
Acute MDMA
Mean Transit Time (sec)
2.5
2.0
1.5
1.0
0.5
0.0
*
Vehicle
Prior MDMA
Capillary Transit Time (sec)
2.5
2.0
1.5
1.0
0.5
0.0
*
*
Vehicle
Prior MDMA
amplitude (arbitrary values)
0.15
0.10
0.05
0.00
*
*
Vehicle
Prior MDMA

(D)

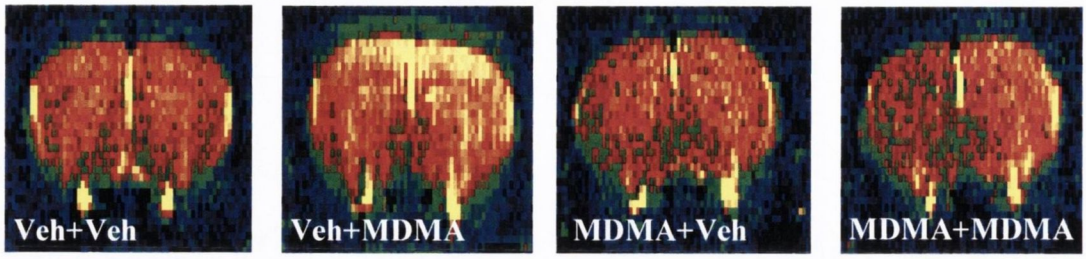


Figure 5.3.1 Prior exposure to MDMA attenuates increased cerebral cortical perfusion induced by acute MDMA challenge

Acute MDMA administration produces a decrease in MTT and CTT with a corresponding increase in signal amplitude in primary (A), secondary motor (B) and somatosensory (C) cortex. Prior MDMA administration attenuates these acute MDMA-induced changes in all three cortical regions. Data are expressed as mean ± SEM (n=8-9). * $p<0.05$; ** $p<0.01$ vs. corresponding vehicle treated control animals (Fisher's LSD *post hoc* test). (D) Representative CBV maps depicting the ability of prior MDMA exposure to attenuate the increase in CBV induced by acute MDMA challenge.

5.3.2 Cortical 5-HT concentration in response to prior MDMA exposure and acute MDMA challenge

ANOVA of cortical 5-HT concentration following repeated MDMA administration (10 mg/kg; i.p., four times daily over 2 days) showed an effect of MDMA pre-treatment [$F_{(1,30)}$=4.4, p<0.05] and an MDMA pre-treatment x acute MDMA challenge interaction [$F_{(1,30)}$=7.97, p<0.01]. *Post hoc* comparisons revealed that acute MDMA challenge produced a decrease in 5-HT concentration 3 hr following administration when compared to vehicle treated controls. In addition 5-HT concentration was reduced in the prior MDMA exposed groups, indicative of long-term 5-HT loss, in comparison to vehicle treated controls (Figure 5.3.2).

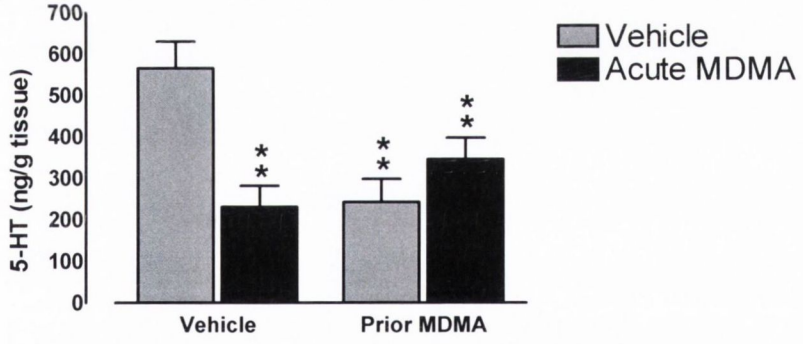


Figure 5.3.2 Cortical 5-HT concentration in response to prior MDMA exposure and acute MDMA challenge

Acute MDMA administration reduces 5-HT concentration in comparison to vehicle treated control animals. Long-term 5-HT depletion is evident 8 weeks following repeated MDMA administration in comparison to vehicle treated controls. Data are

expressed as mean ± SEM (n=8-9). ** $p<0.01$ vs. vehicle treated control animals (Fisher's LSD *post hoc* test).

5.4 Discussion

The results of the present investigation indicate that repeated administration of MDMA (5 mg/kg; i.p., four times daily over 2 days) failed to produce a long-term change in MTT, CTT or signal amplitude, in primary, secondary motor or somatosensory cortex, 8 weeks following drug exposure when compared to vehicle treated controls. As MDMA at 5 mg/kg failed to provoke a reduction in cortical 5-HT concentration, the effects of repeated administration of a higher dose of 10 mg/kg, which induced a long-term reduction in cortical 5-HT concentration, was also assessed. This regime of repeated MDMA administration has previously been reported to cause significant 5-HT loss in the frontal cortex 4 weeks following drug administration (Durkin *et al.*, 2008). Despite the reduction in cortical 5-HT concentration, exposure to the higher dose of MDMA failed to produce a long-term change in the perfusion parameters or signal amplitude when compared to vehicle treated controls. It was concluded therefore that prior exposure to MDMA provokes a long-term reduction in cortical 5-HT concentration which is not associated with a change in cerebral cortical perfusion or blood volume. Long-term 5-HT loss alone may not influence tone of the microvasculature or metabolic demand and consequently cerebral perfusion remains unchanged.

The present findings are in accordance with a previous study in Dark Agouti rats (Ferrington *et al.*, 2006) which reported a lack of change in CBF in any of the brain regions analysed, including the frontal cortex, 3 weeks following administration of a single dose of MDMA (15 mg/kg; i.p.). Long-term 5-HT neurotoxicity was confirmed with a 47% reduction in cortical [^{3}H] paroxetine binding following pre-treatment with

MDMA in comparison to controls. The Dark Agouti strain is particularly sensitive to the effects of MDMA on account of the fact that these animals are poor metabolisers of MDMA. A single dose of MDMA (10 – 15 mg/kg) is required to produce a 30 - 50% depletion of cerebral 5-HT content (Green *et al.,* 2003, for review) which is in contrast to the several doses of MDMA, often of 20 mg/kg or more, which are required to produce a similar degree of 5-HT loss in Wistar, Hooded Lister and Sprague-Dawley rats (Aguirre *et al.,* 1998; Colado *et al.,* 1993; Shankaran & Gudelsky, 1999). O'Shea and colleagues (1998) demonstrated a 40% reduction in cortical 5-HT 1 week following a lower dose of MDMA (4 mg/kg; i.p., twice daily over 4 days) to Dark Agouti rats. A further study assessing long-term cerebral perfusion changes associated with MDMA administration in rats was carried out by van Donkelaar and colleagues (2010) who reported an increase in LCBF in dorsal medial prefrontal cortex, lateral amygdala, septal nucleus, substantia nigra and locus coeruleus of Wistar rats 3 weeks following a repeated MDMA administration regime (20 mg/kg; i.p., twice daily over 4 days) in comparison to vehicle treated controls. A 45% reduction in [^{3}H] paroxetine binding in the frontal cortex of animals treated with MDMA was reported in this study in comparison to controls. Thus differences particular to the strain used, the treatment regime, the dose and route of drug administration, which are contributing factors to the degree of long term 5-HT neurotoxicity may also account for the variation in acute or long-term CBF changes reported with repeated MDMA administration to rats.

Acute MDMA challenge induced a reduction in MTT and CTT and an increase in CBV values in motor and somatosensory cortex in comparison to vehicle treated controls as previously described. The parameters were unchanged in the striatum, thalamus and hippocampus. Prior exposure to MDMA (10 mg/kg), having no effect alone, attenuated

perfusion changes associated with acute MDMA challenge. As previously stated, this attenuation was associated with a long-term reduction in cortical 5-HT concentration, suggesting that MDMA-induced 5-HT loss, while having no effect at baseline, attenuates the response to acute MDMA challenge. Thus a functional deficit develops following prior MDMA exposure in relation to the cerebrovascular and/or neurovascular coupling response to acute MDMA challenge. The results have implications in relation to long-term deficits in the regulation of cerebral perfusion associated with prior MDMA exposure, not basally apparent, but which appears in the face of pharmacological stimulation or challenge.

Prior exposure to MDMA with associated long-term 5-HT loss has been previously reported to diminish the behavioural, physiological and neurochemical response to subsequent MDMA challenge including the 5-HT behavioural syndrome (Shankaran & Gudelsky, 1999; Shankaran *et al.,* 2001), the core body temperature response (Green *et al.,* 2004) and the ability of MDMA to increase extracellular 5-HT concentration (Amato *et al.,* 2007; Shankaran *et al.,* 2001). Ferrington and co-workers (2006) reported decreases in CBF in 12 brain regions analysed, including nucleus accumbens, septal nucleus, hypothalamus, anterior thalamus, nucleus reunions, posterior cingulate and piriform cortex, lateral and medial habenula, superior colliculus, ventral CA1 and ventral subiculum, associated with acute MDMA challenge to rats following prior MDMA exposure 3 weeks earlier. These observations may be regarded as consistent with the findings of the present investigation where prior MDMA exposure diminished raised cortical perfusion associated with acute MDMA challenge.

Given the reduction in response to acute MDMA challenge following prior exposure to the drug associated with long-term cortical 5-HT loss, it is interesting to speculate if the reduction in responsivity to acute challenge may have functional implications. Tests of attention, memory and learning, frontal lobe function, and general intelligence were assessed in 28 recreational ecstasy users (Gouzoulis-Mayfrank *et al.,* 2000). It was reported that performance in simple reaction time tasks of attention were unaffected by MDMA use however, ecstasy users performed worse in more complex attention tasks, in memory and learning tasks and in the tasks of general intelligence. The authors suggested that the cognitive impairments observed may be related to MDMA-induced 5-HT loss as 5-HT is implicated in various cognitive tasks involving memory and speed of information processing (Hasbroucq *et al.,* 1997; Sirvio *et al.,* 1994). Hippocampal dysfunction in current abstinent MDMA users was reported following an associative learning task carried out in conjunction with fMRI (Daumann *et al.,* 2005) and a neuropsychological test battery (Gouzoulis-Mayfrank *et al.,* 2003). The authors suggested that 5-HT loss associated with MDMA use may have a role to play as 5-HT-containing neurons of the median raphe nucleus targeting the hippocampus are critically involved in memory functions.

Impairments in cognition following MDMA administration have since been more widely reported in humans (Daumann *et al.,* 2003; Gouzoulis-Mayfrank *et al.,* 2003; 2005) and in animals (Able *et al.,* 2006; Brevard *et al.,* 2006; Camarasa *et al.,* 2008; Taffe *et al.,* 2001; 2002). Spatial and non-spatial memory were tested using the Morris water maze and novel object recognition tasks respectively in Long Evans rats exposed to MDMA (1 - 15 mg/kg; s.c., twice daily over 4 consecutive days) (Camarasa *et al.,* 2008). Results showed that animals exposed to MDMA (15 mg/kg) showed impairment in the novel object recognition task 72 hr following drug exposure. Taffe and colleagues (2001)

reported an acute cognitive impairment in Rhesus monkeys following a repeated MDMA administration protocol (10 mg/kg; i.m., twice daily over 4 consecutive days) however, no long-term effects of the MDMA regime on memory performance was reported. The authors suggested that differences in testing procedures or sensitivity to the 5-HT depleting effects of MDMA may be responsible for the different effects observed between non-human primates and humans. Despite a growing awareness that MDMA exposure and long-term use may predispose to impairments in cognition, to date there have been no investigations to determine if such changes may associate with changes in cerebral perfusion at a basal performance level or in response to pharmacological or psychological challenge.

It has been suggested that cognitive impairment during, and subsequent to, MDMA exposure may be a vascular, rather than purely neuronal, phenomenon (Ferrington *et al.*, 2006). Long-term depletion of 5-HT following a single exposure to MDMA results in a potential loss of cerebrovascular constrictor tone which may be compounded further by deficits in cerebrovascular autoregulatory capacity associated with acute exposure.

As the incidence of CVA is of concern in relation to MDMA use (Agaba *et al.*, 2002; Auer *et al.*, 2002; Miranda & O'Neill, 2002; Perez *et al.*, 1999; Petitti *et al.*, 1998), repeated exposure to MDMA may predispose to cerebral infarction which would contribute to the emergence of cognitive impairment. It has been suggested that behavioural problems in some ecstasy users during abstinence might be related to 5-HT mediated changes in blood perfusion limited to cortical regions where cerebral SERT binding is most affected (Kish *et al.*, 2010). In addition, it has been suggested that a subtle decline in cognitive capacity may not be noticed by the MDMA users themselves

over a prolonged period of time (Gouzoulis-Mayfrank *et al.*, 2003) and that subjects may continue using ecstasy, leading to a greater cumulative risk for cognitive deficits to progress.

Future clinical studies of MDMA users are likely to be directed towards correlating cognitive decline with small vessel disease and stroke as well as with loss of 5-HT nerve terminals. In this regard btASL MRI will be a useful investigational tool with translational potential for assessment of the long-term effects of MDMA "ecstasy" on cerebral blood perfusion.

Chapter 6

Discussion

Chapter 6: Discussion

6.1 Discussion

In the present investigation, experiments were dedicated firstly to characterise the dose, time and regional effects of MDMA on cerebral perfusion and then attempt to elucidate possible mechanisms underlying the response obtained. The effects observed were accompanied by central 5-HT depletion and, in relation to repeated administration, a long term loss of 5-HT was evident 8 weeks following drug exposure. Such loss however could not be associated with cortical perfusion changes although long-term functional consequences of repeated administration on perfusion were observed following acute MDMA challenge. The effects reported in this investigation must be considered extremely pertinent in light of numerous case reports of cerebrovascular events linked to MDMA use, the unpredictable nature of ecstasy associated toxicity in humans and the likely long-term functional consequences, which are likely to emerge as a consequence of damage to 5-HT neurons.

Many previous studies have investigated the cerebrovascular changes associated with both acute and long-term MDMA exposure in animals (Ferrington *et al.,* 2006; Quate *et al.,* 2004; Rosa-Neto *et al.,* 2004; van Donkelaar *et al.,* 2010) and humans (Chang *et al.,* 2000; Finsterer *et al.,* 2003; Reneman *et al.,* 2000). It has been suggested that the cerebrovascular changes associated with long-term exposure to MDMA may lead to a vascular, and not solely neuronal, associated cognitive decline (Ferrington *et al.,* 2006). MDMA-induced cognitive declines have been widely reported in animals (Able *et al.,* 2006; Brevard *et al.,* 2006; Camarasa *et al.,* 2008; Taffe *et al.,* 2001; 2003). Kalechstein and colleagues (2007)

carried out a meta-analysis of the changes in neurocognition associated with MDMA exposure. Studies were separated into two cohorts based on their inclusion/exclusion criteria, a relatively stringent criteria that comprised 11 studies and a relatively lenient criteria that comprised 23 studies. Subjects were tested in a series of neurocognitive tasks including attention/concentration, verbal learning and memory, nonverbal learning and memory, motor/psychomotor speed and executive systems functioning. Findings from the review indicated that, in both study cohorts, MDMA exposure was associated with poorer performance in each of the neurocognitive tasks assessed.

The quantitative method btASL MRI as developed by Kelly and co-workers (2009) has been applied throughout this project to investigate the CBF and CBV changes associated with exposure to the psychoactive drug of abuse MDMA "ecstasy" in a rodent model. There are published reports of btASL fMRI in assessment of perfusion changes associated with varied manipulations in animal models under hypoxic conditions. Wegener and colleagues (2008), under isoflourane anaesthesia, demonstrated an increase in CBF in sensory/auditory cortex, hippocampus, thalamus, caudate putamen and globus pallidus in Wistar rats following a 14 min hypoxic incident and 5 days later a significant decrease in CBF was reported, in all brain regions, when the animals were re-exposed to hypoxia. Forepaw stimulation, in Wistar rats, has previously been reported to induce a decrease in MTT and CTT and an increase in relative CBV of labelled water ($rCBV_{lw}$) in the somatosensory cortex, indicative of increased perfusion in this area (Griffin *et al.,* 2010; Kelly *et al.,* 2010). Previous experiments have used ASL to investigate CBF changes following exposure to cocaine (Chen *et al.,* 2001; Luo *et al.,* 2009). ASL has advantages over BOLD when conducting pharmacological MRI (phMRI) studies, including its suitability for studying slow changes in brain function and its ability to offer quantitative

CBF measurements both at rest and during activation, which is critical for separating drug effects on baseline brain function and challenge-induced activation. In addition, ASL offers improved visualisation of the orbitofrontal, inferior temporal, and limbic regions that are linked to major neurotransmitter systems. One of the primary limitations of using ASL for phMRI studies is that some drugs may lead to systemic cardiovascular effects and a promising approach to circumvent confounding factors in phMRI is to estimate drug-induced changes in cerebral metabolic rate of oxygen ($CMRO_2$) through combined ASL and BOLD scanning. In addition, another challenge associated with the use of this approach is the lower sensitivity and image cover of existing ASL methods compared with BOLD fMRI.

phMRI is increasingly being used to speed the translation of discovery and development of new drugs, from the laboratory to the clinic, and ASL perfusion MRI is being used more widely as an alternative and complementary tool to BOLD fMRI (Detre *et al.,* 2009). ASL MRI measurements, in humans, have previously been reported to correlate closely with ^{15}O water PET CBF both at rest (Ye *et al.,* 2000) and during task activation (Feng *et al.,* 2004). ASL may be considered to be an advantageous fMRI technique over the available alternatives as it is entirely non-invasive and can be repeated as often as is required to track the effects of pharmacological challenges over minutes, hours, days and weeks (Wang *et al.,* 2011, for review). In addition, ASL offers quantitative CBF measurements both at rest and during task activation, which is critical for separating drug effects on baseline brain function and task-induced activation (Liau *et al.,* 2008).

7T MRI with laboratory animals requires that the animals are sedated or anaesthetised in advance of the scanning procedures and this is an important factor to be considered when

quantifying cerebral perfusion changes. It has previously been reported that some inhalational anaesthetics (nitrous oxide, halothane and isoflourane) cause cerebral vasodilatation and therefore tend to increase CBF (van Hemelrijck *et al.,* 1993) and investigators have attempted to address this issue by using injectable anaesthetics while conducting their experiments (Griffin *et al.,* 2010; Kelly *et al.,* 2010). Using a forepaw stimulus to investigate perfusion and activation of the somatosensory cortex with btASL MRI in rats, Kelly and co-workers used the sedative medetomidine, an α_2-adrenergic receptor agonist, whereas Griffin and colleagues used the anaesthetic propofol. MTT and CTT values were similar in the control groups of both investigations suggesting that the choice of anaesthetic had no apparent influence on these perfusion parameters. By contrast, reported $rCBV_{lw}$ values between studies were lower when medetomidine was used and Griffin and colleagues (2010) suggest that this mode of hypnosis may mediate vasoconstriction which is sustained during forepaw stimulation and activation of the somatosensory cortex. By contrast, it is of interest to note that one of the side effects of propofol is that it modulates blood vessel tone leading to vasodilatation (Bentley *et al.,* 1989; Ririe *et al.,* 2001). Signal amplitude values, in control animals, from the experiments described in the current body of work on perfusion changes associated with MDMA are in line with those reported by Kelly and colleagues (2010), where medetomidine was used. There is evidence therefore that anaesthetic choice may influence btASL parameters and in particular signal amplitude and CBV determinations which are apparent at baseline and are likely to influence functional response to varied stimuli. Careful consideration should be given to the choice of anaesthetic or sedative used when carrying out these experiments. In the current investigation btASL experiments used the combination of ketamine/xylazine for anaesthesia. Ketamine is classified as an N-methyl D-aspartate (NMDA) receptor antagonist while xylazine acts as an agonist at α_2-adrenergic receptors. It has previously

been reported that ketamine increases rCBF (Rowland *et al.,* 2010) and that xylazine decreases CBF (Lei *et al.,* 2001). These findings indicate that there are possible roles for ketamine and xylazine in mediating changes in CBF.

It is clear that btASL can be utilised for the assessment of cerebral perfusion changes following physiological stimulation and pharmacological challenge however, validation of the method is necessary if it is to be considered further as a translational tool. A number of previous studies have assessed CBF and CBV changes associated with MDMA exposure using PET (Banks *et al.,* 2008; Buchert *et al.,* 2004; Gould *et al.,* 2011; Li *et al.,* 2010; McCann *et al.,* 2005; Reneman *et al.,* 2006) and SPECT (Cowan, 2007; de Win *et al.,* 2004; 2008; Finsterer *et al.,* 2003; Reneman *et al.,* 2001; 2006) imaging in both humans and animals. In addition to this, the *ex-vivo* [^{14}C] iodoantipyrine autoradiography approach has previously been used to assess for rCBF changes associated with MDMA exposure in animal studies (Ferrington *et al.,* 2006; Quate *et al.,* 2004; van Donkelaar *et al.,* 2010). It would be of interest to test if the CBF and CBV changes reported using btASL MRI are matched using parallel alternative techniques under similar experimental conditions. Such validation would serve to confirm the translational value of btASL MRI in animals for informing clinical investigations and in relation to the potential use of the technique in clinical environments. A major limitation in relation to the translational potential of pre-clinical investigations remains however, the necessity for use of anaesthesia or sedation when compared to human MRI, where scanning may be performed without such a requirement.

When considering the translational relevance of MDMA-induced changes to cerebral perfusion in the laboratory setting it is important to consider the animal model that is used.

The issue of interspecies scaling has been raised (McCann & Ricaurte, 2001) and addressed previously in terms of appropriate dosing and neurotoxicity (de la Torre & Farré, 2004; Green *et al.,* 2003, for review; Green *et al.,* 2009). In general the effects of MDMA are consistent across species, with the notable exception of the mouse where it appears to have a more profound effect on dopamine that 5-HT (Colado *et al.,* 2004). From a toxicity viewpoint, the primate has been found to be 4 to 8 times more sensitive to the toxic effects of MDMA compared to the rodent (Ricaurte *et al.,* 1988). It has been argued that doses used in experimental animals are too high to be relevant to humans, but there is a counter-argument that a differential susceptibility to the effects of the drug exists between species. The application of interspecies scaling developed over 20 years ago (Mordenti, 1986) goes some way towards reconciling the apparent discrepancy in drug dosage. In order to extrapolate doses used in animal studies to those used in man, interspecies scaling has provided evidence to indicate that an approximately five-fold higher MDMA dose in animals will produce similar results in man e.g. the dose of 20 mg/kg in rats becomes equivalent to 4 mg/kg in man (Green *et al.,* 2009). Green and colleagues, using dose-plasma concentration response curves, reported that a four-fold higher dose in animals is required to produce a similar peak blood plasma exposure to that seen in humans. In addition, it has been reported that MDMA (1.4 mg/kg) increases oral temperature by 0.6°C in humans (Farré *et al.,* 2007) and a similar increase is reported following MDMA (5 mg/kg; i.p.) administration to rats (Colado *et al.,* 1995), an approximate four-fold increase in dose. It has been reported that differences in MDMA metabolism among animal species might account for different sensitivities to its neurotoxic effects (de la Torre & Farré, 2004). MDMA metabolites, HHMA and HMMA, are found in both rats and humans at different levels following MDMA administration, indicating that although metabolic pathways are similar there are nevertheless relevant differences. Careful consideration of

the dose of MDMA is required when addressing the potential clinical implications or relevance of findings ascertained from the use of animal models of MDMA abuse.

Numerous pharmacological challenges were carried out during this set of investigations to elucidate a role for 5-HT and/or dopamine in mediating the MDMA-induced increases in cerebral perfusion. A singular role for these neurotransmitters in mediating this response was not observed, although the possibility remains that both 5-HT and dopamine may work in concert to effect changes associated with MDMA administration. In addition it is important to consider other putative contributing factors. Adrenergic receptors have previously been implicated in the thermoregulatory changes (Bexis & Docherty, 2005; 2006; 2009; Docherty & Green, 2010) and the locomotor response (Selken & Nichols, 2007) associated with MDMA administration to rats. In addition there is evidence to suggest that peripheral vascular and related blood pressure changes associated with MDMA are mediated by adrenergic receptors (McDaid & Docherty, 2001). A role for α_2-adrenergic, and possibly α_1-adrenergic, receptors in mediating the initial increase in blood pressure following MDMA (5 mg/kg; i.v.) administration to rats has been described. The increase in blood pressure recorded 1 min following administration was largely α_1-adrenoceptor mediated while the sustained decrease in blood pressure that followed was as a result of α_2-adrenoceptor activation. MDMA has also been reported to have significant $\alpha_{2A/D}$-agonist actions, in anaesthetised mice, which contribute to the rapid decline from an increased to a decreased blood pressure response (Vandeputte & Docherty, 2002). Authors also reported the likelihood that these receptors were centrally located. These results provide evidence for a role of α-adrenergic receptors in mediating peripheral vascular changes associated with MDMA exposure. As α-adrenergic receptors have previously been reported to be located on cerebral microvessels (Bryan *et al.,* 1996; Yokoo *et al.,* 2000) it is not

unreasonable to propose a possible role for α_1- and/or α_2-adrenergic receptors in mediating the increased cerebral perfusion response associated with acute MDMA exposure.

6.2 Future Directions

The findings presented in this thesis have yielded a number of important leads for future research as outlined below:

(1) The mechanism of MDMA-induced increases in cerebral perfusion needs to be further examined, with particular focus on the role of α_1- and α_2-adrenoceptors in mediating this response.

(2) The mechanism of MDMA-induced increases in cerebral perfusion needs to be further examined, with particular focus on the role of non-monoaminergic mechanisms in mediating this response, for example the role that NO and/or arachidonic acid metabolites may have to play.

(3) Validation of the btASL method as a tool for assessing CBF and CBV changes needs to be addressed, with particular focus on the use of PET and SPECT imaging.

(4) To measure blood pressure in tandem with assessing cerebral perfusion changes to elucidate whether or not MDMA has the ability to disrupt autoregulation i.e. the ability of the cerebrovasculature to resist peripheral changes in blood pressure.

(5) Coupling of the ASL measures to monoamine release using *in vivo* microdialysis, as this would help to clarify a role for monoamines in the MDMA-induced cerebral perfusion changes observed with btASL MRI. Such an approach has previously been adopted by others when investigating the effects of amphetamine

administration to rats on cerebral perfusion (Chen *et al.,* 2004; 2010; Choi *et al.,* 2006).

(6) To determine if the effects of MDMA generalise to other amphetamines, or drugs of abuse such as cocaine, would be of interest. The model developed could be used further to investigate any putative interactions between MDMA and other commonly used illicit or licit drugs such as ethanol or nicotine.

(7) To employ btASL in recreational MDMA users to determine if effects observed in the rodent model may be identified in drug users.

(8) To assess for cognitive changes associated with long-term MDMA exposure in tandem with determination of CBF and CBV measures and further assessment post mortem to identify potential pathological markers associated with perfusion changes related to prior exposure to MDMA.

References

Able JA, Gudelsky GA, Vorhees CV & Williams MT. (2006). 3,4-Methylenedioxymethamphetamine in adult rats produces deficits in path integration and spatial reference memory. *Biol Psychiatry* **59,** 1219-1226.

Agaba EA, Lynch RM, Baskaran A & Jackson T. (2002). Massive intracerebral hematoma and extradural hematoma in amphetamine abuse. *Am J Emerg Med* **20,** 55-57.

Aguirre N, Ballaz S, Lasheras B & Del Rio J. (1998). MDMA ('Ecstasy') enhances 5-HT1A receptor density and 8-OH-DPAT-induced hypothermia: blockade by drugs preventing 5-hydroxytryptamine depletion. *Eur J Pharmacol* **346,** 181-188.

Alsop DC & Detre JA. (1996). Reduced transit-time sensitivity in noninvasive magnetic resonance imaging of human cerebral blood flow. *J Cereb Blood Flow Metab* **16,** 1236-1249.

Amato JL, Bankson MG & Yamamoto BK. (2007). Prior exposure to chronic stress and MDMA potentiates mesoaccumbens dopamine release mediated by the 5-HT(1B) receptor. *Neuropsychopharmacology* **32,** 946-954.

Andresen J, Shafi NI & Bryan RM, Jr. (2006). Endothelial influences on cerebrovascular tone. *J Appl Physiol* **100,** 318-327.

Auer J, Berent R, Weber T, Lassnig E & Eber B. (2002). Subarachnoid haemorrhage with "Ecstasy" abuse in a young adult. *Neurol Sci* **23,** 199-201.

Bai F, Jones DC, Lau SS & Monks TJ. (2001). Serotonergic neurotoxicity of 3,4-(+/-)-methylenedioxyamphetamine and 3,4-(+/-)-methylendioxymethamphetamine (ecstasy) is potentiated by inhibition of gamma-glutamyl transpeptidase. *Chem Res Toxicol* **14,** 863-870.

Bai F, Lau SS & Monks TJ. (1999). Glutathione and N-acetylcysteine conjugates of alpha-methyldopamine produce serotonergic neurotoxicity: possible role in methylenedioxyamphetamine-mediated neurotoxicity. *Chem Res Toxicol* **12,** 1150-

1157.

Banks ML, Czoty PW, Gage HD, Bounds MC, Garg PK, Garg S & Nader MA. (2008). Effects of cocaine and MDMA self-administration on serotonin transporter availability in monkeys. *Neuropsychopharmacology* **33,** 219-225.

Bankson MG, Breier JM & Yamamoto BK. (2005). MDMA causes long-term increases in blood brain barrier permeability.

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

Battaglia G, Brooks BP, Kulsakdinun C & De Souza EB. (1988). Pharmacologic profile of MDMA (3,4-methylenedioxymethamphetamine) at various brain recognition sites. *Eur J Pharmacol* **149,** 159-163.

Battaglia G, Yeh SY, O'Hearn E, Molliver ME, Kuhar MJ & De Souza EB. (1987). 3,4-Methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: quantification of neurodegeneration by measurement of [3H]paroxetine-labeled serotonin uptake sites. *J Pharmacol Exp Ther* **242,** 911-916.

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

Beckmann CF, Jenkinson M, Woolrich MW, Behrens TE, Flitney DE, Devlin JT & Smith SM. (2006). Applying FSL to the FIAC data: model-based and model-free analysis of voice and sentence repetition priming. *Hum Brain Mapp* **27,** 380-391.

Bentley GN, Gent JP & Goodchild CS. (1989). Vascular effects of propofol: smooth muscle relaxation in isolated veins and arteries. *J Pharm Pharmacol* **41,** 797-798.

Bergers G & Song S. (2005). The role of pericytes in blood-vessel formation and maintenance. *Neuro Oncol* **7,** 452-464.

Berntman L, Carlsson C, Hagerdal M & Siesjo BK. (1978). Circulatory and metabolic effects in the brain induced by amphetamine sulphate. *Acta Physiol Scand* **102,** 310-323.

Bexis S & Docherty JR. (2005). Role of alpha2A-adrenoceptors in the effects of MDMA on body temperature in the mouse. *Br J Pharmacol* **146,** 1-6.

Bexis S & Docherty JR. (2006). Effects of MDMA, MDA and MDEA on blood pressure, heart rate, locomotor activity and body temperature in the rat involve alpha-adrenoceptors. *Br J Pharmacol* **147,** 926-934.

Bexis S & Docherty JR. (2009). Role of alpha 1- and beta 3-adrenoceptors in the modulation by SR59230A of the effects of MDMA on body temperature in the mouse. *Br J Pharmacol* **158,** 259-266.

Bouchelet I, Case B, Olivier A & Hamel E. (2000). No contractile effect for 5-HT1D and 5-HT1F receptor agonists in human and bovine cerebral arteries: similarity with human coronary artery. *Br J Pharmacol* **129,** 501-508.

Brevard ME, Meyer JS, Harder JA & Ferris CF. (2006). Imaging brain activity in conscious monkeys following oral MDMA ("ecstasy"). *Magn Reson Imaging* **24,** 707-714.

Broadley KJ. (2010). The vascular effects of trace amines and amphetamines. *Pharmacol Ther* **125,** 363-375.

Bryan RM, Jr., Eichler MY, Swafford MW, Johnson TD, Suresh MS & Childres WF. (1996). Stimulation of alpha 2 adrenoceptors dilates the rat middle cerebral artery. *Anesthesiology* **85,** 82-90.

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.

Cadet JL & Brannock C. (1998). Free radicals and the pathobiology of brain dopamine systems. *Neurochem Int* **32,** 117-131.

Camarasa J, Marimon JM, Rodrigo T, Escubedo E & Pubill D. (2008). Memantine prevents the cognitive impairment induced by 3,4-methylenedioxymethamphetamine in rats. *Eur J Pharmacol* **589,** 132-139.

Cao F & Leung LS. (1992). Effect of atropine and PCPA on the behavioral modulation of paired-pulse response in the hippocampal CA1 region. *Brain Res* **576,** 339-342.

Capela JP, Macedo C, Branco PS, Ferreira LM, Lobo AM, Fernandes E, Remiao F, Bastos ML, Dirnagl U, Meisel A & Carvalho F. (2007). Neurotoxicity mechanisms of thioether ecstasy metabolites. *Neuroscience* **146,** 1743-1757.

Capela JP, Macedo C, Branco PS, Ferreira LM, Lobo AM, Fernandes E, Remiao F, Bastos ML, Dirnagl U, Meisel A & Carvalho F. (2007). Neurotoxicity mechanisms of thioether ecstasy metabolites. *Neuroscience* **146,** 1743-1757.

Carlsson A. (1975). Drugs acting through dopamine release. *Pharmacol Ther B* **1,** 401-405.

Carmignoto G & Gomez-Gonzalo M. (2010). The contribution of astrocyte signalling to neurovascular coupling. *Brain Res Rev* **63,** 138-148.

Carvalho M, Remiao F, Milhazes N, Borges F, Fernandes E, Carvalho F & Bastos ML. (2004). The toxicity of N-methyl-alpha-methyldopamine to freshly isolated rat hepatocytes is prevented by ascorbic acid and N-acetylcysteine. *Toxicology* **200,** 193-203.

Carvalho M, Remiao F, Milhazes N, Borges F, Fernandes E, Monteiro Mdo C, Goncalves MJ, Seabra V, Amado F, Carvalho F & Bastos ML. (2004). Metabolism is required for the expression of ecstasy-induced cardiotoxicity in vitro. *Chem Res Toxicol* **17,** 623-632.

Cauli B & Hamel E. (2010). Revisiting the role of neurons in neurovascular coupling. *Front Neuroenergetics* **2,** 9.

Chang L, Grob CS, Ernst T, Itti L, Mishkin FS, Jose-Melchor R & Poland RE. (2000). Effect of ecstasy [3,4-methylenedioxymethamphetamine (MDMA)] on cerebral blood flow: a co-registered SPECT and MRI study. *Psychiatry Res* **98,** 15-28.

Che S, Johnson M, Hanson GR & Gibb JW. (1995). Body temperature effect on methylenedioxymethamphetamine-induced acute decrease in tryptophan hydroxylase activity. *Eur J Pharmacol* **293,** 447-453.

Chen YC, Choi JK, Andersen SL, Rosen BR & Jenkins BG. (2005). Mapping dopamine D2/D3 receptor function using pharmacological magnetic resonance imaging. *Psychopharmacology (Berl)* **180,** 705-715.

Chen YC, Mandeville JB, Nguyen TV, Talele A, Cavagna F & Jenkins BG. (2001). Improved mapping of pharmacologically induced neuronal activation using the IRON technique with superparamagnetic blood pool agents. *J Magn Reson Imaging* **14,** 517-524.

Chen YI, Choi JK, Xu H, Ren J, Andersen SL & Jenkins BG. (2010). Pharmacologic neuroimaging of the ontogeny of dopamine receptor function. *Dev Neurosci* **32,** 125-138.

Choi JK, Chen YI, Hamel E & Jenkins BG. (2006). Brain hemodynamic changes mediated by dopamine receptors: Role of the cerebral microvasculature in dopamine-mediated neurovascular coupling. *Neuroimage* **30,** 700-712.

Chu T, Kumagai Y, DiStefano EW & Cho AK. (1996). Disposition of methylenedioxymethamphetamine and three metabolites in the brains of different rat strains and their possible roles in acute serotonin depletion. *Biochem Pharmacol* **51,** 789-796.

Cohen Z, Bonvento G, Lacombe P & Hamel E. (1996). Serotonin in the regulation of brain microcirculation. *Prog Neurobiol* **50,** 335-362.

Cohen Z, Ehret M, Maitre M & Hamel E. (1995). Ultrastructural analysis of tryptophan hydroxylase immunoreactive nerve terminals in the rat cerebral cortex and hippocampus: their associations with local blood vessels. *Neuroscience* **66,** 555-569.

Colado MI, Murray TK & Green AR. (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 MI, O'Shea E & Green AR. (2004). Acute and long-term effects of MDMA on cerebral dopamine biochemistry and function. *Psychopharmacology (Berl)* **173,** 249-263.

Colado MI, Williams JL & Green AR. (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. *Br J Pharmacol* **115,** 1281-1289.

Cole JC & Sumnall HR. (2003). Altered states: the clinical effects of Ecstasy. *Pharmacol Ther* **98,** 35-58.

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

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

Crespi D, Gobbi M & Mennini T. (1997). 5-HT3 serotonin hetero-receptors inhibit [3H]acethylcholine release in rat cortical synaptosomes. *Pharmacol Res* **35,** 351-354.

Cryan JF, Harkin A, Naughton M, Kelly JP & Leonard BE. (2000). Characterization of D-fenfluramine-induced hypothermia: evidence for multiple sites of action. *Eur J Pharmacol* **390,** 275-285.

Dafters RI. (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 RI & 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.

Daumann J, Fimm B, Willmes K, Thron A & Gouzoulis-Mayfrank E. (2003). Cerebral activation in abstinent ecstasy (MDMA) users during a working memory task: a functional magnetic resonance imaging (fMRI) study. *Brain Res Cogn Brain Res* **16,** 479-487.

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

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

de la Torre R, Farre M, Roset PN, Lopez CH, Mas M, Ortuno J, Menoyo E, Pizarro N, Segura J & Cami J. (2000). Pharmacology of MDMA in humans. *Ann N Y Acad Sci* **914,** 225-237.

de la Torre R, Farre M, Roset PN, Pizarro N, Abanades S, Segura M, Segura J & Cami J. (2004). Human pharmacology of MDMA: pharmacokinetics, metabolism, and disposition. *Ther Drug Monit* **26,** 137-144.

De Silva RN & Harries DP. (1992). Misuse of ecstasy. *BMJ* **305,** 310.

De Souza EB & Battaglia G. (1989). Effects of MDMA and MDA on brain serotonin neurons: evidence from neurochemical and autoradiographic studies. *NIDA Res Monogr* **94,** 196-222.

De Souza I, Kelly J.P., Harkin A.J. & B.E. L. (1997). An appraisal of the pharmacological and toxicological effects of a single oral administration of 3,4-methylenedioxymethamphetamine (MDMA) in the rat *Pharmacology and Toxicology* **80,** 207-210.

de Win MM, de Jeu RA, de Bruin K, Habraken JB, Reneman L, Booij J & den Heeten GJ. (2004). Validity of in vivo [123I]beta-CIT SPECT in detecting MDMA-induced neurotoxicity in rats. *Eur Neuropsychopharmacol* **14,** 185-189.

de Win MM, Jager G, Booij J, Reneman L, Schilt T, Lavini C, Olabarriaga SD, den Heeten GJ & van den Brink W. (2008). Sustained effects of ecstasy on the human brain: a prospective neuroimaging study in novel users. *Brain* **131,** 2936-2945.

de Win MM, Reneman L, Jager G, Vlieger EJ, Olabarriaga SD, Lavini C, Bisschops I, Majoie CB, Booij J, den Heeten GJ & van den Brink W. (2007). A prospective cohort study on sustained effects of low-dose ecstasy use on the brain in new ecstasy users. *Neuropsychopharmacology* **32,** 458-470.

Detre JA, Wang J, Wang Z & Rao H. (2009). Arterial spin-labeled perfusion MRI in basic

and clinical neuroscience. *Curr Opin Neurol* **22,** 348-355.

Di Matteo V, Di Giovanni G, Pierucci M & Esposito E. (2008). Serotonin control of central dopaminergic function: focus on in vivo microdialysis studies. *Prog Brain Res* **172,** 7-44.

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

Doly S, Valjent E, Setola V, Callebert J, Herve D, Launay JM & Maroteaux L. (2008). Serotonin 5-HT2B receptors are required for 3,4-methylenedioxymethamphetamine-induced hyperlocomotion and 5-HT release in vivo and in vitro. *J Neurosci* **28,** 2933-2940.

Dore-Duffy P. (2008). Pericytes: pluripotent cells of the blood brain barrier. *Curr Pharm Des* **14,** 1581-1593.

Downing J. (1986). The psychological and physiological effects of MDMA on normal volunteers. *J Psychoactive Drugs* **18,** 335-340.

Drake CT & Iadecola C. (2007). The role of neuronal signaling in controlling cerebral blood flow. *Brain Lang* **102,** 141-152.

Dumont GJ, Schoemaker RC, Touw DJ, Sweep FC, Buitelaar JK, van Gerven JM & Verkes RJ. (2009). Acute psychomotor effects of MDMA and ethanol (co-) administration over time in healthy volunteers. *J Psychopharmacol* **24,** 155-164.

Durkin S, Prendergast A & Harkin A. (2008). Reduced efficacy of fluoxetine following MDMA ("Ecstasy")-induced serotonin loss in rats. *Prog Neuropsychopharmacol Biol Psychiatry* **32,** 1894-1901.

Edvinsson L, Hardebo JE, MacKenzie ET & Stewart M. (1977). Dual action of serotonin

on pial arterioles in situ and the effect of propranolol on the response. *Blood Vessels* **14,** 366-371.

Edvinsson L, McCulloch J & Sharkey J. (1985). Vasomotor responses of cerebral arterioles in situ to putative dopamine receptor agonists. *Br J Pharmacol* **85,** 403-410.

Egerton A, Mehta MA, Montgomery AJ, Lappin JM, Howes OD, Reeves SJ, Cunningham VJ & Grasby PM. (2009). The dopaminergic basis of human behaviors: A review of molecular imaging studies. *Neurosci Biobehav Rev* **33,** 1109-1132.

Elhusseiny A & Hamel E. (2001). Sumatriptan elicits both constriction and dilation in human and bovine brain intracortical arterioles. *Br J Pharmacol* **132,** 55-62.

El-Mallakh RS & Abraham HD. (2007). MDMA (Ecstasy). *Ann Clin Psychiatry* **19,** 45-52.

Esteban B, O'Shea E, Camarero J, Sanchez V, Green AR & Colado MI. (2001). 3,4-Methylenedioxymethamphetamine induces monoamine release, but not toxicity, when administered centrally at a concentration occurring following a peripherally injected neurotoxic dose. *Psychopharmacology (Berl)* **154,** 251-260.

Farre M, Abanades S, Roset PN, Peiro AM, Torrens M, O'Mathuna B, Segura M & de la Torre R. (2007). Pharmacological interaction between 3,4-methylenedioxymethamphetamine (ecstasy) and paroxetine: pharmacological effects and pharmacokinetics. *J Pharmacol Exp Ther* **323,** 954-962.

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

Feng CM, Narayana S, Lancaster JL, Jerabek PA, Arnow TL, Zhu F, Tan LH, Fox PT & Gao JH. (2004). CBF changes during brain activation: fMRI vs. PET. *Neuroimage* **22,** 443-446.

Ferrington L, Kirilly E, McBean DE, Olverman HJ, Bagdy G & Kelly PA. (2006). Persistent cerebrovascular effects of MDMA and acute responses to the drug. *Eur J Neurosci* **24,** 509-519.

Finsterer J, Stollberger C, Steger C & Kroiss A. (2003). Long lasting impaired cerebral blood flow after ecstasy intoxication. *Psychiatry Clin Neurosci* **57,** 221-225.

Florence G, Bonnier R, Plagnes D, Pierard C, Satabin P, Peres M & Lagarde D. (2000). Effect of modafinil on cerebral blood flow of anaesthetised rats. Comparison with amphetamine. *Exp Brain Res* **135,** 552-556.

Freese TE, Miotto K & Reback CJ. (2002). The effects and consequences of selected club drugs. *J Subst Abuse Treat* **23,** 151-156.

Gamma A, Buck A, Berthold T, Liechti ME & Vollenweider FX. (2000). 3,4-Methylenedioxymethamphetamine (MDMA) modulates cortical and limbic brain activity as measured by [H(2)(15)O]-PET in healthy humans. *Neuropsychopharmacology* **23,** 388-395.

Gerra G, Zaimovic A, Ferri M, Zambelli U, Timpano M, Neri E, Marzocchi GF, 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.

Girouard H & Iadecola C. (2006). Neurovascular coupling in the normal brain and in hypertension, stroke, and Alzheimer disease. *J Appl Physiol* **100,** 328-335.

Gledhill JA, Moore DF, Bell D & Henry JA. (1993). Subarachnoid haemorrhage associated

with MDMA abuse. *J Neurol Neurosurg Psychiatry* **56,** 1036-1037.

Golan D.E., Tashjian A.H., Armstrong E.J. & A.W. A. (2008). *Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy*. Lippincott Williams & Wilkins, Baltimore.

Golozoubova V, Strauss F & Malmlof K. (2006). Locomotion is the major determinant of sibutramine-induced increase in energy expenditure. *Pharmacol Biochem Behav* **83,** 517-527.

Gong CL, Lin NN & Kuo JS. (2002). Glutamatergic and serotonergic mechanisms in the dorsal facial area for common carotid artery blood flow control in the cat. *Auton Neurosci* **101,** 85-90.

Gonzalez FJ, Skoda RC, Kimura S, Umeno M, Zanger UM, Nebert DW, Gelboin HV, Hardwick JP & Meyer UA. (1988). Characterization of the common genetic defect in humans deficient in debrisoquine metabolism. *Nature* **331,** 442-446.

Gordon CJ, Watkinson WP, O'Callaghan JP & Miller DB. (1991). Effects of 3,4-methylenedioxymethamphetamine on autonomic thermoregulatory responses of the rat. *Pharmacol Biochem Behav* **38,** 339-344.

Gould RW, Gage HD, Banks ML, Blaylock BL, Czoty PW & Nader MA. (2011). Differential effects of cocaine and MDMA self-administration on cortical serotonin transporter availability in monkeys. *Neuropharmacology* **61,** 245-251.

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

Gouzoulis-Mayfrank E, Fischermann T, Rezk M, Thimm B, Hensen G & Daumann J. (2005). Memory performance in polyvalent MDMA (ecstasy) users who continue or discontinue MDMA use. *Drug Alcohol Depend* **78,** 317-323.

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

Green AR, Gabrielsson J, Marsden CA & Fone KC. (2009). MDMA: on the translation from rodent to human dosing. *Psychopharmacology (Berl)* **204,** 375-378.

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

Green AR, O'Shea E & Colado MI. (2004). A review of the mechanisms involved in the acute MDMA (ecstasy)-induced hyperthermic response. *Eur J Pharmacol* **500,** 3-13.

Griffin KM, Blau CW, Kelly ME, O'Herlihy C, O'Connell PR, Jones JF & Kerskens CM. (2010). Propofol allows precise quantitative arterial spin labelling functional magnetic resonance imaging in the rat. *Neuroimage* **51,** 1395-1404.

Gudelsky GA & Yamamoto BK. (2008). Actions of 3,4-methylenedioxymethamphetamine (MDMA) on cerebral dopaminergic, serotonergic and cholinergic neurons. *Pharmacol Biochem Behav* **90,** 198-207.

Guillemin GJ & Brew BJ. (2004). Microglia, macrophages, perivascular macrophages, and pericytes: a review of function and identification. *J Leukoc Biol* **75,** 388-397.

Hamel E. (2006). Perivascular nerves and the regulation of cerebrovascular tone. *J Appl Physiol* **100,** 1059-1064.

Hamilton NB, Attwell D & Hall CN. (2010). Pericyte-mediated regulation of capillary diameter: a component of neurovascular coupling in health and disease. *Front Neuroenergetics* **2**.

Hansen-Schwartz J, Hoel NL, Xu CB, Svendgaard NA & Edvinsson L. (2003). Subarachnoid hemorrhage-induced upregulation of the 5-HT1B receptor in cerebral arteries in rats. *J Neurosurg* **99,** 115-120.

Hanyu S, Ikeguchi K, Imai H, Imai N & Yoshida M. (1995). Cerebral infarction associated with 3,4-methylenedioxymethamphetamine ('Ecstasy') abuse. *Eur Neurol* **35,** 173.

Harkin A, Connor TJ, Mulrooney J, Kelly JP & Leonard BE. (2001). Prior exposure to methylenedioxyamphetamine (MDA) induces serotonergic loss and changes in spontaneous exploratory and amphetamine-induced behaviors in rats. *Life Sci* **68,** 1367-1382.

Harries DP & De Silva R. (1992). 'Ecstasy' and intracerebral haemorrhage. *Scott Med J* **37,** 150-152.

Hasbroucq T, Rihet P, Blin O & Possamai CA. (1997). Serotonin and human information processing: fluvoxamine can improve reaction time performance. *Neurosci Lett* **229,** 204-208.

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

Haydon PG & Carmignoto G. (2006). Astrocyte control of synaptic transmission and neurovascular coupling. *Physiol Rev* **86,** 1009-1031.

Haydon PG & Carmignoto G. (2006). Astrocyte control of synaptic transmission and neurovascular coupling. *Physiol Rev* **86,** 1009-1031.

Heistad DD, Marcus ML, Sandberg S & Abboud FM. (1977). Effect of sympathetic nerve stimulation on cerebral blood flow and on large cerebral arteries of dogs. *Circ Res*

41, 342-350.

Hemeryck A & Belpaire FM. (2002). Selective serotonin reuptake inhibitors and cytochrome P-450 mediated drug-drug interactions: an update. *Curr Drug Metab* **3,** 13-37.

Henry JA. (1992). Ecstasy and the dance of death. *BMJ* **305,** 5-6.

Henry JA, Jeffreys KJ & Dawling S. (1992). Toxicity and deaths from 3,4-methylenedioxymethamphetamine ("ecstasy"). *Lancet* **340,** 384-387.

Huettel SA SA, McCarthy G. (2008). *Functional magnetic reonance imaging.* Freeman WH, New York.

Hughes JC, McCabe M & Evans RJ. (1993). Intracranial haemorrhage associated with ingestion of 'ecstasy'. *Arch Emerg Med* **10,** 372-374.

Iadecola C. (2004). Neurovascular regulation in the normal brain and in Alzheimer's disease. *Nat Rev Neurosci* **5,** 347-360.

Jahng GH, Weiner MW & Schuff N. (2007). Improved arterial spin labeling method: applications for measurements of cerebral blood flow in human brain at high magnetic field MRI. *Med Phys* **34,** 4519-4525.

Jones DC, Duvauchelle C, Ikegami A, Olsen CM, Lau SS, de la Torre R & Monks TJ. (2005). Serotonergic neurotoxic metabolites of ecstasy identified in rat brain. *J Pharmacol Exp Ther* **313,** 422-431.

Jones DC, Lau SS & Monks TJ. (2004). Thioether metabolites of 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine inhibit human serotonin transporter (hSERT) function and simultaneously stimulate dopamine uptake into hSERT-expressing SK-N-MC cells. *J Pharmacol Exp Ther* **311,** 298-306.

Kaku DA & Lowenstein DH. (1990). Emergence of recreational drug abuse as a major risk factor for stroke in young adults. *Ann Intern Med* **113,** 821-827.

Kalant H. (2001). The pharmacology and toxicology of "ecstasy" (MDMA) and related drugs. *CMAJ* **165,** 917-928.

Kalechstein AD, De La Garza R, 2nd, Mahoney JJ, 3rd, Fantegrossi WE & Newton TF. (2007). MDMA use and neurocognition: a meta-analytic review. *Psychopharmacology (Berl)* **189,** 531-537.

Kamouchi M, Ago T & Kitazono T. (2011). Brain pericytes: emerging concepts and functional roles in brain homeostasis. *Cell Mol Neurobiol* **31,** 175-193.

Kamouchi M, Ago T & Kitazono T. (2011). Brain pericytes: emerging concepts and functional roles in brain homeostasis. *Cell Mol Neurobiol* **31,** 175-193.

Kamouchi M, Ago T, Kuroda J & Kitazono T. (2004). The Possible Roles of Brain Pericytes in Brain Ischemia and Stroke. *Cell Mol Neurobiol.*

Kaumann AJ, Parsons AA & Brown AM. (1993). Human arterial constrictor serotonin receptors. *Cardiovasc Res* **27,** 2094-2103.

Kelly ME, Blau CW, Griffin KM, Gobbo OL, Jones JF & Kerskens CM. (2010). Quantitative functional magnetic resonance imaging of brain activity using bolus-tracking arterial spin labeling. *J Cereb Blood Flow Metab* **30,** 913-922.

Kelly ME, Blau CW & Kerskens CM. (2009). Bolus-tracking arterial spin labelling: theoretical and experimental results. *Phys Med Biol* **54,** 1235-1251.

Kelly PA, Ritchie IM, Sangra M, Cursham MJ, Dickson EM, Kelly B, Neilson FP, Reidy MJ & Stevens MC. (1994). Hyperaemia in rat neocortex produced by acute exposure to methylenedioxymethamphetamine. *Brain Res* **665,** 315-318.

Kish SJ. (2002). How strong is the evidence that brain serotonin neurons are damaged in human users of ecstasy? *Pharmacol Biochem Behav* **71,** 845-855.

Kish SJ, Furukawa Y, Ang L, Vorce SP & Kalasinsky KS. (2000). Striatal serotonin is depleted in brain of a human MDMA (Ecstasy) user. *Neurology* **55,** 294-296.

Kish SJ, Lerch J, Furukawa Y, Tong J, McCluskey T, Wilkins D, Houle S, Meyer J, Mundo E, Wilson AA, Rusjan PM, Saint-Cyr JA, Guttman M, Collins DL, Shapiro C, Warsh JJ & Boileau I. (2010). Decreased cerebral cortical serotonin transporter binding in ecstasy users: a positron emission tomography/[(11)C]DASB and structural brain imaging study. *Brain* **133,** 1779-1797.

Kobari M, Fukuuchi Y, Tomita M, Tanahashi N, Konno S & Takeda H. (1993). Effects of sumatriptan on the cerebral intraparenchymal microcirculation in the cat. *Br J Pharmacol* **110,** 1445-1448.

Koch S & Galloway MP. (1997). MDMA induced dopamine release in vivo: role of endogenous serotonin. *J Neural Transm* **104,** 135-146.

Koehler RC, Roman RJ & Harder DR. (2009). Astrocytes and the regulation of cerebral blood flow. *Trends Neurosci* **32,** 160-169.

Kohnomi S, Suemaru K, Kawasaki H & Araki H. (2008). Effect of aripiprazole on 5-HT2 receptor-mediated wet-dog shake responses and disruption of prepulse inhibition in rats. *J Pharmacol Sci* **106,** 645-650.

Kreth K, Kovar K, Schwab M & Zanger UM. (2000). Identification of the human cytochromes P450 involved in the oxidative metabolism of "Ecstasy"-related designer drugs. *Biochem Pharmacol* **59,** 1563-1571.

Krimer LS, Muly EC, 3rd, Williams GV & Goldman-Rakic PS. (1998). Dopaminergic regulation of cerebral cortical microcirculation. *Nat Neurosci* **1,** 286-289.

Kuo JS, Li HT, Lin NN, Yang CS & Cheng FC. (1999). Dorsal facial area of cat medulla; 5-HT2 action on glutamate release in regulating common carotid blood flow. *Neurosci Lett* **266,** 137-140.

Lei H, Grinberg O, Nwaigwe CI, Hou HG, Williams H, Swartz HM & Dunn JF. (2001). The effects of ketamine-xylazine anesthesia on cerebral blood flow and oxygenation observed using nuclear magnetic resonance perfusion imaging and electron paramagnetic resonance oximetry. *Brain Res* **913,** 174-179.

Lester SJ, Baggott M, Welm S, Schiller NB, Jones RT, Foster E & Mendelson J. (2000). Cardiovascular effects of 3,4-methylenedioxymethamphetamine. A double-blind, placebo-controlled trial. *Ann Intern Med* **133,** 969-973.

Li HT, Chen WY, Liu L, Yang CS, Cheng FC, Chai CY & Kuo JS. (1996). The dorsal facial area of the medulla in cats: inhibitory action of serotonin on glutamate release in regulating common carotid blood flow. *Neurosci Lett* **210,** 193-196.

Li IH, Huang WS, Shiue CY, Huang YY, Liu RS, Chyueh SC, Hu SH, Liao MH, Shen LH, Liu JC & Ma KH. Study on the neuroprotective effect of fluoxetine against MDMA-induced neurotoxicity on the serotonin transporter in rat brain using micro-PET. *Neuroimage* **49,** 1259-1270.

Liau J, Perthen JE & Liu TT. (2008). Caffeine reduces the activation extent and contrast-to-noise ratio of the functional cerebral blood flow response but not the BOLD response. *Neuroimage* **42,** 296-305.

Liechti ME, Baumann C, Gamma A & Vollenweider FX. (2000). Acute psychological effects of 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") are attenuated by the serotonin uptake inhibitor citalopram. *Neuropsychopharmacology* **22,** 513-521.

Liechti ME, Saur MR, Gamma A, Hell D & Vollenweider FX. (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 ME & Vollenweider FX. (2000). Acute psychological and physiological effects of MDMA ("Ecstasy") after haloperidol pretreatment in healthy humans. *Eur Neuropsychopharmacol* **10,** 289-295.

Liechti ME & Vollenweider FX. (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.

Luo Z, Yuan Z, Tully M, Pan Y & Du C. (2009). Quantification of cocaine-induced cortical blood flow changes using laser speckle contrast imaging and Doppler optical coherence tomography. *Appl Opt* **48,** D247-255.

Malberg JE, Sabol KE & Seiden LS. (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.

Mamounas LA & Molliver ME. (1988). Evidence for dual serotonergic projections to neocortex: axons from the dorsal and median raphe nuclei are differentially vulnerable to the neurotoxin p-chloroamphetamine (PCA). *Exp Neurol* **102,** 23-36.

Marston HM, Reid ME, Lawrence JA, Olverman HJ & Butcher SP. (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 Torre R, Roset PN, 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.

Mayerhofer A, Kovar KA & Schmidt WJ. (2001). Changes in serotonin, dopamine and

noradrenaline levels in striatum and nucleus accumbens after repeated administration of the abused drug MDMA in rats. *Neurosci Lett* **308,** 99-102.

McBean DE, Sharkey J, Ritchie IM & Kelly PA. (1991). Cerebrovascular and functional consequences of 5-HT1A receptor activation. *Brain Res* **555,** 159-163.

McCann UD & Ricaurte GA. (2001). Caveat emptor: editors beware. *Neuropsychopharmacology* **24,** 333-336.

McCann UD & Ricaurte GA. (2004). Amphetamine neurotoxicity: accomplishments and remaining challenges. *Neurosci Biobehav Rev* **27,** 821-826.

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

McCann UD, Szabo Z, Scheffel U, Dannals RF & Ricaurte GA. (1998). Positron emission tomographic evidence of toxic effect of MDMA ("Ecstasy") on brain serotonin neurons in human beings. *Lancet* **352,** 1433-1437.

McCann UD, Szabo Z, Seckin E, Rosenblatt P, Mathews WB, Ravert HT, Dannals RF & Ricaurte GA. (2005). Quantitative PET studies of the serotonin transporter in MDMA users and controls using [11C]McN5652 and [11C]DASB. *Neuropsychopharmacology* **30,** 1741-1750.

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

McEvoy AW, Kitchen ND & Thomas DG. (2000). Intracerebral haemorrhage and drug abuse in young adults. *Br J Neurosurg* **14,** 449-454.

McKenna DJ & Peroutka SJ. (1990). Neurochemistry and neurotoxicity of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). *J Neurochem* **54,** 14-22.

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

Milner TA, Reis DJ & Giuliano R. (1996). Afferent sources of substance P in the C1 area of the rat rostral ventrolateral medulla. *Neurosci Lett* **205,** 37-40.

Miranda J & O'Neill D. (2002). Stroke associated with amphetamine use. *Ir Med J* **95,** 281-282.

Mokler DJ, Commissaris RL, Warner MR & Rech RH. (1983). Blockade of the behavioral effects of lysergic acid diethylamide, 2,5-dimethoxy-4-methylamphetamine, quipazine and lisuride by 5-hydroxytryptamine antagonists. *J Pharmacol Exp Ther* **227,** 557-562.

Molliver ME, Berger UV, Mamounas LA, Molliver DC, O'Hearn E & Wilson MA. (1990). Neurotoxicity of MDMA and related compounds: anatomic studies. *Ann N Y Acad Sci* **600,** 649-661; discussion 661-644.

Mordenti J. (1986). Man versus beast: pharmacokinetic scaling in mammals. *J Pharm Sci* **75,** 1028-1040.

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

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

Nilsson T, Longmore J, Shaw D, Olesen IJ & Edvinsson L. (1999). Contractile 5-HT1B receptors in human cerebral arteries: pharmacological characterization and

localization with immunocytochemistry. *Br J Pharmacol* **128,** 1133-1140.

Obrocki J, Schmoldt A, Buchert R, Andresen B, Petersen K & Thomasius R. (2002). Specific neurotoxicity of chronic use of ecstasy. *Toxicol Lett* **127,** 285-297.

O'Cain PA, Hletko SB, Ogden BA & Varner KJ. (2000). Cardiovascular and sympathetic responses and reflex changes elicited by MDMA. *Physiol Behav* **70,** 141-148.

O'Loinsigh ED, Boland G, Kelly JP & O'Boyle KM. (2001). Behavioural, hyperthermic and neurotoxic effects of 3,4-methylenedioxymethamphetamine analogues in the Wistar rat. *Prog Neuropsychopharmacol Biol Psychiatry* **25,** 621-638.

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

Osredkar D & Krzan M. (2009). Expression of serotonin receptor subtypes in rat brain and astrocyte cell cultures: an age and tissue-dependent process *Periodicum Biologorum* **111,** 129-135.

Paris JM & Cunningham KA. (1992). Lack of serotonin neurotoxicity after intraraphe microinjection of (+)-3,4-methylenedioxymethamphetamine (MDMA). *Brain Res Bull* **28,** 115-119.

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

Parrott AC. (2011). MDMA and temperature: A review of the thermal effects of 'Ecstasy' in humans. *Drug Alcohol Depend.*

Parsons AA. (1991). 5-HT receptors in human and animal cerebrovasculature. *Trends*

Pharmacol Sci **12,** 310-315.

Paulson OB, Hasselbalch SG, Rostrup E, Knudsen GM & Pelligrino D. (2010). Cerebral blood flow response to functional activation. *J Cereb Blood Flow Metab* **30,** 2-14.

Peppiatt CM, Howarth C, Mobbs P & Attwell D. (2006). Bidirectional control of CNS capillary diameter by pericytes. *Nature* **443,** 700-704.

Perez JA, Jr., Arsura EL & Strategos S. (1999). Methamphetamine-related stroke: four cases. *J Emerg Med* **17,** 469-471.

Peroutka SJ, Newman H & Harris H. (1988). Subjective effects of 3,4-methylenedioxymethamphetamine in recreational users. *Neuropsychopharmacology* **1,** 273-277.

Petitti DB, Sidney S, Quesenberry C & Bernstein A. (1998). Stroke and cocaine or amphetamine use. *Epidemiology* **9,** 596-600.

Piper BJ, Fraiman JB, Owens CB, Ali SF & Meyer JS. (2008). Dissociation of the neurochemical and behavioral toxicology of MDMA ('Ecstasy') by citalopram. *Neuropsychopharmacology* **33,** 1192-1205.

Price LH, Malison RT, McDougle CJ, McCance-Katz EF, Owen KR & Heninger GR. (1997). Neurobiology of tryptophan depletion in depression: effects of m-chlorophenylpiperazine (mCPP). *Neuropsychopharmacology* **17,** 342-350.

Puerta E, Hervias I, Goni-Allo B, Zhang SF, Jordan J, Starkov AA & Aguirre N. (2010). Methylenedioxymethamphetamine inhibits mitochondrial complex I activity in mice: a possible mechanism underlying neurotoxicity. *Br J Pharmacol* **160,** 233-245.

Quate L, McBean DE, Ritchie IM, Olverman HJ & Kelly PA. (2004). Acute methylenedioxymethamphetamine administration: effects on local cerebral blood

flow and glucose utilisation in the Dark Agouti rat. *Psychopharmacology (Berl)* **173,** 287-295.

Raichle ME, Hartman BK, Eichling JO & Sharpe LG. (1975). Central noradrenergic regulation of cerebral blood flow and vascular permeability. *Proc Natl Acad Sci U S A* **72,** 3726-3730.

Ren J, Xu H, Choi JK, Jenkins BG & Chen YI. (2009). Dopaminergic response to graded dopamine concentration elicited by four amphetamine doses. *Synapse* **63,** 764-772.

Reneman L, de Win MM, van den Brink W, Booij J & den Heeten GJ. (2006). Neuroimaging findings with MDMA/ecstasy: technical aspects, conceptual issues and future prospects. *J Psychopharmacol* **20,** 164-175.

Reneman L, Habraken JB, Majoie CB, Booij J & den Heeten GJ. (2000). MDMA ("Ecstasy") and its association with cerebrovascular accidents: preliminary findings. *AJNR Am J Neuroradiol* **21,** 1001-1007.

Reneman L, Majoie CB, Habraken JB & den Heeten GJ. (2001). Effects of ecstasy (MDMA) on the brain in abstinent users: initial observations with diffusion and perfusion MR imaging. *Radiology* **220,** 611-617.

Rho JM & Storey TW. (2001). Molecular ontogeny of major neurotransmitter receptor systems in the mammalian central nervous system: norepinephrine, dopamine, serotonin, acetylcholine, and glycine. *J Child Neurol* **16,** 271-280; discussion 281.

Ricaurte GA, DeLanney LE, Irwin I & Langston JW. (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 GA, Yuan J & McCann UD. (2000). (+/-)3,4-Methylenedioxymethamphetamine ('Ecstasy')-induced serotonin neurotoxicity: studies in animals. *Neuropsychobiology* **42,** 5-10.

Ririe DG, Lundell JC & Neville MJ. (2001). Direct effects of propofol on myocardial and vascular tissue from mature and immature rats. *J Cardiothorac Vasc Anesth* **15,** 745-749.

Roberts C, Price GW & Jones BJ. (1997). The role of 5-HT(1B/1D) receptors in the modulation of 5-hydroxytryptamine levels in the frontal cortex of the conscious guinea pig. *Eur J Pharmacol* **326,** 23-30.

Rosa-Neto P, Olsen AK, Gjedde A, Watanabe H & Cumming P. (2004). MDMA-evoked changes in cerebral blood flow in living porcine brain: correlation with hyperthermia. *Synapse* **53,** 214-221.

Rothman RB, Baumann MH, Dersch CM, Romero DV, Rice KC, Carroll FI & Partilla JS. (2001). Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. *Synapse* **39,** 32-41.

Rowland LM, Beason-Held L, Tamminga CA & Holcomb HH. (2010). The interactive effects of ketamine and nicotine on human cerebral blood flow. *Psychopharmacology (Berl)* **208,** 575-584.

Russo KE, Hall W, Chi OZ, Sinha AK & Weiss HR. (1991). Effect of amphetamine on cerebral blood flow and capillary perfusion. *Brain Res* **542,** 43-48.

Sanchez V, Camarero J, Esteban B, Peter MJ, Green AR & Colado MI. (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.

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

Scheffel U, Szabo Z, Mathews WB, Finley PA, Dannals RF, Ravert HT, Szabo K, Yuan J & Ricaurte GA. (1998). In vivo detection of short- and long-term MDMA neurotoxicity--a positron emission tomography study in the living baboon brain. *Synapse* **29,** 183-192.

Schmidt CJ, Levin JA & 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 CJ & Taylor VL. (1988). Direct central effects of acute methylenedioxymethamphetamine on serotonergic neurons. *Eur J Pharmacol* **156,** 121-131.

Selken J & Nichols DE. (2007). Alpha1-adrenergic receptors mediate the locomotor response to systemic administration of (+/-)-3,4-methylenedioxymethamphetamine (MDMA) in rats. *Pharmacol Biochem Behav* **86,** 622-630.

Semple DM, Ebmeier KP, Glabus MF, O'Carroll RE & Johnstone EC. (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 GA. (1998). Effect of 3,4-methylenedioxymethamphetamine (MDMA) on hippocampal dopamine and serotonin. *Pharmacol Biochem Behav* **61,** 361-366.

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

Shankaran M, Yamamoto BK & Gudelsky GA. (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.

Sharma HS & Ali SF. (2008). Acute administration of 3,4-methylenedioxymethamphetamine induces profound hyperthermia, blood-brain barrier disruption, brain edema formation, and cell injury. *Ann N Y Acad Sci* **1139,** 242-258.

Sirvio J, Riekkinen P, Jr., Jakala P & Riekkinen PJ. (1994). Experimental studies on the role of serotonin in cognition. *Prog Neurobiol* **43,** 363-379.

Solowij N, Hall W & Lee N. (1992). Recreational MDMA use in Sydney: a profile of 'Ecstacy' users and their experiences with the drug. *Br J Addict* **87,** 1161-1172.

Spatt J, Glawar B & Mamoli B. (1997). A pure amnestic syndrome after MDMA ("ecstasy") ingestion. *J Neurol Neurosurg Psychiatry* **62,** 418-419.

Squier MV, Jalloh S, Hilton-Jones D & Series H. (1995). Death after ecstasy ingestion: neuropathological findings. *J Neurol Neurosurg Psychiatry* **58,** 756.

Stachowicz K, Chojnacka-Wojcik E, Klak K & Pilc A. (2007). Anxiolytic-like effect of group III mGlu receptor antagonist is serotonin-dependent. *Neuropharmacology* **52,** 306-312.

Steinbusch HW. (1981). Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. *Neuroscience* **6,** 557-618.

Steinkellner T, Freissmuth M, Sitte HH & Montgomery T. (2011). The ugly side of amphetamines: short- and long-term toxicity of 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy'), methamphetamine and D-amphetamine. *Biol Chem* **392,** 103-115.

Taffe MA, Davis SA, Yuan J, Schroeder R, Hatzidimitriou G, Parsons LH, Ricaurte GA & Gold LH. (2002). Cognitive performance of MDMA-treated rhesus monkeys:

sensitivity to serotonergic challenge. *Neuropsychopharmacology* **27,** 993-1005.

Taffe MA, Huitron-Resendiz S, Schroeder R, Parsons LH, Henriksen SJ & Gold LH. (2003). MDMA exposure alters cognitive and electrophysiological sensitivity to rapid tryptophan depletion in rhesus monkeys. *Pharmacol Biochem Behav* **76,** 141-152.

Taffe MA, Weed MR, Davis S, Huitron-Resendiz S, Schroeder R, Parsons LH, Henriksen SJ & Gold LH. (2001). Functional consequences of repeated (+/-)3,4-methylenedioxymethamphetamine (MDMA) treatment in rhesus monkeys. *Neuropsychopharmacology* **24,** 230-239.

Tan H, Maldjian JA, Pollock JM, Burdette JH, Yang LY, Deibler AR & Kraft RA. (2009). A fast, effective filtering method for improving clinical pulsed arterial spin labeling MRI. *J Magn Reson Imaging* **29,** 1134-1139.

Teggin AF. (1992). Ecstasy--a dangerous drug. *S Afr Med J* **81,** 431-432.

Thomas DL, Lythgoe MF, Pell GS, Calamante F & Ordidge RJ. (2000). The measurement of diffusion and perfusion in biological systems using magnetic resonance imaging. *Phys Med Biol* **45,** R97-138.

Thompson MR, Li KM, Clemens KJ, Gurtman CG, Hunt GE, Cornish JL & McGregor IS. (2004). Chronic fluoxetine treatment partly attenuates the long-term anxiety and depressive symptoms induced by MDMA ('Ecstasy') in rats. *Neuropsychopharmacology* **29,** 694-704.

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.

Tucker GT, Lennard MS, Ellis SW, Woods HF, Cho AK, Lin LY, Hiratsuka A, Schmitz DA & Chu TY. (1994). The demethylenation of methylenedioxymethamphetamine ("ecstasy") by debrisoquine hydroxylase (CYP2D6). *Biochem Pharmacol* **47,** 1151-

1156.

Ullmer C, Schmuck K, Kalkman HO & Lubbert H. (1995). Expression of serotonin receptor mRNAs in blood vessels. *FEBS Lett* **370,** 215-221.

Vaarmann A, Gandhi S & Abramov AY. (2010). Dopamine induces Ca2+ signaling in astrocytes through reactive oxygen species generated by monoamine oxidase. *J Biol Chem* **285,** 25018-25023.

van Donkelaar EL, Kelly PA, Dawson N, Blokland A, Prickaerts J, Steinbusch HW & Ferrington L. (2010). Acute tryptophan depletion potentiates 3,4-methylenedioxymethamphetamine-induced cerebrovascular hyperperfusion in adult male Wistar rats. *J Neurosci Res* **88,** 1557-1568.

Van Hemelrijck J, Waets P, Van Aken H, Lacroix H, Nevelsteen A & Suy R. (1993). Blood pressure management during aortic surgery: urapidil compared to isosorbide dinitrate. *J Cardiothorac Vasc Anesth* **7,** 273-278.

Vanattou-Saifoudine N, McNamara R & Harkin A. (2010). Caffeine promotes dopamine D1 receptor-mediated body temperature, heart rate and behavioural responses to MDMA ('ecstasy'). *Psychopharmacology (Berl)* **211,** 15-25.

Vanattou-Saifoudine N, McNamara R & Harkin A. (2010). Mechanisms mediating the ability of caffeine to influence MDMA ('Ecstasy')-induced hyperthermia in rats. *Br J Pharmacol* **160,** 860-877.

Vandeputte C & Docherty JR. (2002). Vascular actions of 3,4-methylenedioxymethamphetamine in alpha(2A/D)-adrenoceptor knockout mice. *Eur J Pharmacol* **457,** 45-49.

Verheyden SL, Henry JA & Curran HV. (2003). Acute, sub-acute and long-term subjective consequences of 'ecstasy' (MDMA) consumption in 430 regular users. *Hum Psychopharmacol* **18,** 507-517.

Volkow ND, Fowler JS, Wang GJ, Baler R & Telang F. (2009). Imaging dopamine's role in drug abuse and addiction. *Neuropharmacology* **56 Suppl 1,** 3-8.

Volkow ND, Wang GJ, Kollins SH, Wigal TL, Newcorn JH, Telang F, Fowler JS, Zhu W, Logan J, Ma Y, Pradhan K, Wong C & Swanson JM. (2009). Evaluating dopamine reward pathway in ADHD: clinical implications. *JAMA* **302,** 1084-1091.

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

Volterra A & Meldolesi J. (2005). Astrocytes, from brain glue to communication elements: the revolution continues. *Nat Rev Neurosci* **6,** 626-640.

Wallace TL, Gudelsky GA & Vorhees CV. (2001). Neurotoxic regimen of methamphetamine produces evidence of behavioral sensitization in the rat. *Synapse* **39,** 1-7.

Wang DJ, Chen Y, Fernandez-Seara MA & Detre JA. (2011). Potentials and challenges for arterial spin labeling in pharmacological magnetic resonance imaging. *J Pharmacol Exp Ther* **337,** 359-366.

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

Wegener S & Wong EC. (2008). Longitudinal MRI studies in the isoflurane-anesthetized rat: long-term effects of a short hypoxic episode on regulation of cerebral blood flow as assessed by pulsed arterial spin labelling. *NMR Biomed* **21,** 696-703.

Winstock AR, Griffiths P & Stewart D. (2001). Drugs and the dance music scene: a survey

of current drug use patterns among a sample of dance music enthusiasts in the UK. *Drug Alcohol Depend* **64,** 9-17.

Yamamoto BK & Raudensky J. (2008). The role of oxidative stress, metabolic compromise, and inflammation in neuronal injury produced by amphetamine-related drugs of abuse. *J Neuroimmune Pharmacol* **3,** 203-217.

Ye FQ, Berman KF, Ellmore T, Esposito G, van Horn JD, Yang Y, Duyn J, Smith AM, Frank JA, Weinberger DR & McLaughlin AC. (2000). H(2)(15)O PET validation of steady-state arterial spin tagging cerebral blood flow measurements in humans. *Magn Reson Med* **44,** 450-456.

Yokoo H, Kobayashi H, Minami S, Shiraishi S, Yamamoto R, Yanagita T, Tsuchiya K, Mohri M & Wada A. (2000). alpha(1)-Adrenergic receptor subtypes in rat cerebral microvessels. *Brain Res* **878,** 183-187.

VIII Appendix

1. Solutions used

Phosphate-buffered saline

NaCl	100 mM
Na_2HPO_4	80 mM
NaH_2PO_4	20 mM

Distilled water

2. IDL scripts for btASL analysis

1. OPENBOLUS.PRO

```
function openbolus,matrix,runs,ntimes,echo

all=intarr(matrix,matrix/2,runs,ntimes,echo)
label=intarr(matrix,matrix/2,runs/2,ntimes)
control=intarr(matrix,matrix/2,runs/2,ntimes)

for i=0,ntimes-1 do begin
all(*,*,*,i,*)=openrecs(matrix,runs,echo)
      for j=0,runs/2-1 do begin
      label(*,*,j,i)=all(*,*,j,i)
      end
      for j=runs/2,runs-1 do begin
      control(*,*,j-11,i)=all(*,*,j,i)
      end
totallabel=total(label,4)
totalcontrol=total(control,4)
diff_norm=(totalcontrol-totallabel)/totalcontrol
diff=totalcontrol-totallabel
control_max=totalcontrol(*,*,7)
label_max=totallabel(*,*,7)
end

openw,1,'\control_max\'
writeu,1,control_max
close,1

openw,1,'\label_max\'
writeu,1,label_max
close,1

openw,1,'\diff_norm\'
writeu,1,diff_norm
close,1

openw,1,'\diff\'
writeu,1,diff
close,1

return,diff_norm
end
```

2. OPENRECS.PRO

```
function openrecs,matrix,runs,echo

;read file
path=dialog_pickfile(Path="")
procno_dir=findfile(path)
print,procno_dir
      openr,1,procno_dir
      info=fstat(1)
      help,info,/struct
      im=intarr(float(info.size/2))
        readu,1,im
      close,1

;create matrix
sim=intarr(matrix/2,matrix,runs,echo)
help,sim
for j=0, ((runs)-1) do begin
for k=0,     echo-1 do begin
for i=0,(matrix-1) do begin
      iy=float(i)
      jy=echo*float(j)+float(k)
      jj=float(j)
      ky=float(k)
      sim(0:matrix/2-
1,iy,jj,ky)=im(((jy*matrix/2*matrix)+iy*matrix/2):(jy*matrix*matrix/2+(iy
+1)*matrix/2-1))
end
tvscl,sim(0:63,0:63,j,k)
print,j,k
;wait,0.1
end
end

return,sim

end
```

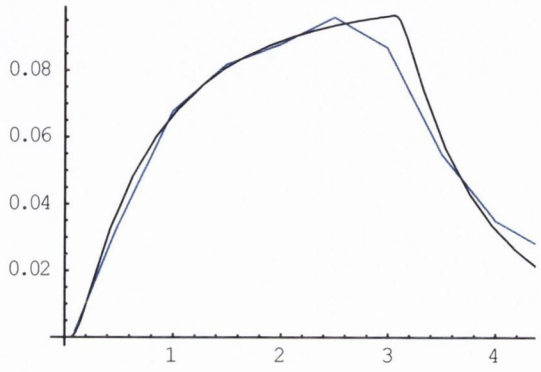
3. MAKEPIC.PRO

```
pro makepic,min=min,max=max,filename=filename
on_error,2

if n_elements(filename) eq 0 then $
  message, 'Must input filename'
if n_elements(min) eq 0 then min=0.0
if n_elements(max) eq 0 then max=0.25

device,decomposed=0
loadct,4

file=dialog_pickfile(path="")

data=fltarr(128,64,11,/nozero)
openr,lun,file,/get_lun
readu,lun,data
free_lun,lun

slice=data[*,*,6]
slice=reverse(slice,2)/50000.0
pic=rebin(slice,256,256,/sample)

window,/free,xsize=356,ysize=256
tv,bytscl(pic,min=min,max=max)
colorbar, range=[min,max], /vertical, divisions=4, $
  color=fsc_color('white'), format='(F0.2)'

cd, "C:\Documents and Settings\rouinej\Desktop\Rep Admin (5
mgkg)\Rat_74\"
void=tvread(/tiff, /overwrite_prompt,filename=filename)


return
end
```

3. Mathematica script for btASL analysis

```
T1=1.7;

R1=1/T1;

tau=3.0;

x={0.1,0.5,1.0,1.5,2.0,2.5,2.99,3.5,4.0,4.5,5.0};

model=Piecewise[{{      a*Exp[b]*(Erfc[(1/(2*Sqrt[t]))*Sqrt[b^2/c]-
Sqrt[(c+R1)*t]]*Exp[-
Sqrt[(b^2)/c]*Sqrt[c+R1]]),t<3.0},{a*Exp[b]*(Erfc[(1/(2*Sqrt[t]))*
Sqrt[b^2/c]-Sqrt[(c+R1)*t]]*Exp[-Sqrt[(b^2)/c]*Sqrt[c+R1]]-
Erfc[(1/(2*Sqrt[t-tau]))*Sqrt[b^2/c]-Sqrt[(c+R1)*(t-tau)]]*Exp[-
Sqrt[(b^2)/c]*Sqrt[c+R1]]),t≥3.0}}]

\[Piecewise]{
  {a ~b Sqrt[b^2/c] Sqrt[0.588235 c]  Erfc[Sqrt[b^2/c]/(2 Sqrt[t])-
Sqrt[0.588235 c t]], t<3.},
  {a ©b (-~Sqrt[b^2/c] Sqrt[0.588235 c]  Erfc[Sqrt[b^2/c]/(2 Sqrt[3. t])-
Sqrt[0.588235 c t 3. t]]+~Sqrt[b^2/c] Sqrt[0.588235 c]  Erfc[Sqrt[b^2/c]/(2
Sqrt[t])-Sqrt[0.588235 c t]]), t≥3.}
 }

y1={0.002,0.034,0.068,0.082,0.088,0.096,0.087,0.055,0.035,0.026,0.
019};

Clear[a,b,c,t]

dataPairs=Transpose[{x,y1}];

solution=FindFit[dataPairs,Norm[model],{{a,0.081},{b,0.641},{c,0.1
78}},{t},MaxIterations→10000,AccuracyGoal→1]

{a→0.0854882,b→0.50237,c→0.184602}
```

```
Show[ListPlot[dataPairs,PlotJoined→True,PlotStyle→{PointSize[0.05
],Hue[0.6]},DisplayFunction→Identity],Plot[model/.solution,{t,0,5}
,DisplayFunction→Identity],DisplayFunction→$DisplayFunction]
```




```
MTT=solution[[2,2]]/(2*solution[[3,2]])
1.36068
CTT=1/(4*solution[[3,2]])
1.35427
a=solution[[1,2]]
0.0990833
b=solution[[2,2]]
0.497037
c=solution[[3,2]]
0.130158
fit=Table[model,{t,0,5,0.25}]
```

IX Publications

MDMA "Ecstasy" increases cerebral cortical perfusion determined by bolus-tracking arterial spin labelling (btASL) MRI
J. Rouine, O. Gobbo, M. Campbell, V. Gigliucci, I. Ogden, K. McHugh Smith, B. Behan, D. Byrne, M. Kelly, C. Blau, C. Kerskens and A. Harkin.
Submitted to *Neuropsychopharmacology*

Investigation of the role of 5-HT and dopamine in mediating increased cortical perfusion following MDMA "Ecstasy"
J. Rouine, M. Kelly, C. Jennings-Murphy, P. Duffy, C Blau, C. Kerskens and A. Harkin.
To be submitted to *Psychopharmacology*

Investigation of the long-term effects of repeated MDMA "Ectsasy" exposure on cerebral cortical perfusion with btASL MRI in rats
J. Rouine, C. Jennings-Murphy, P. Duffy, C. Kerskens and A. Harkin.
To be submitted to *Neuropharmacology*

Published Abstracts

Perfusion magnetic resonance imaging using spin labelling of arterial water shows increased cortical blood flow following methylenedioxymethamphetamine (MDMA;"ecstasy") administration to rats
J. Rouine, B. Behan, M. Kelly, C. Blau, O. Gobbo, C. Kerskens and A. Harkin.
Irish Journal of Medical Science, 2010, Vol 179, Supplement 3, S108.

Methylenedioxymethamphetamine (MDMA;"ecstasy") induced alterations in cerebral blood perfusion determined by arterial spin labelling coupled to magnetic resonance

imaging

J. Rouine, D. Byrne, B. Behan, M. Kelly, C. Blau, O. Gobbo, C. Kerskens and A. Harkin.
FENS Abstr. vol 5, 059.29, 2010.

Cortical blood perfusion following MDMA ("ecstasy") administration in rats determined by spin labelling of arterial water coupled to magnetic resonance imaging

J. Rouine, D. Byrne, B. Behan, M. Kelly, C. Blau, O. Gobbo, C. Kerskens and A. Harkin.
Journal of Psychopharmacology, 2010, Vol 24(8), Supplement 3, A37.

Serotonin related changes to cerebral blood flow and cerebral blood volume determined by bolus-tracking arterial spin labelling magnetic resonance imaging

J. Rouine, C. Jennings-Murphy, C. Kerskens, A. Harkin.
Journal of Psychpharmacology, 2011, Vol 25(8), Supplement 3, A58.

Investigation of cerebral perfusion changes following MDMA "Ecstasy" administration in an animal model using bolus-tracking arterial spin labelling MRI **Jennifer Rouine**

The recreational drug of abuse 3,4-methylenedioxymethamphetamine (MDMA; Ecstasy) carries a risk of cerebrovascular accidents (CVA) that may relate to the role of serotonin (5-HT) and/or dopamine in the regulation of cerebrovascular tone. Recent advances in magnetic resonance imaging (MRI) have enabled measurement of cerebral blood perfusion using contrast agent-free approaches such as bolus-tracking arterial spin labeling (btASL). This investigation assessed changes in cerebral perfusion following systemic MDMA administration to rats using btASL MRI. Adult male Wistar rats were administered MDMA (5 or 20 mg/kg; i.p.) or saline, anaesthetised 1, 3 or 24 hours later and a high resolution anatomical scan followed by a continuous ASL (cASL) sequence was conducted using a 7 Tesla MRI scanner. Perfusion-weighted images were generated by subtraction of labelled from control images and experimental data was fitted to a quantitative model of cerebral perfusion to generate mean transit time (MTT), capillary transit time (CTT) and signal amplitude. MTT and CTT are inversely proportional to cerebral blood flow (CBF) and CBF squared respectively, and signal amplitude is proportional to cerebral blood volume (CBV). MDMA induced a reduction in MTT and CTT and an increase in signal amplitude in primary motor, secondary motor and somatosensory cortex 1 and 3 hours following administration. Such effects were not obtained in sub-cortical regions. The acute effects of MDMA on cerebral perfusion may go some way towards providing a mechanism to explain the occurrence of CVA in vulnerable recreational ecstasy users.

MDMA (20 mg/kg) provoked qualitatively similar effects to the 5-HT releasing drug fenfluramine (10 mg/kg) but not to the 5-HT_2 receptor agonist DOI (1 mg/kg). Depletion of central 5-HT produced a similar effect to that observed with MDMA-induced cortical 5-HT depletion. As 5-HT promotes vasoconstriction predominantly, a loss of the vasoconstrictive action of 5-HT might account for the increase in perfusion observed. Pre-treatment with the non selective 5-HT receptor antagonist metergoline (4 mg/kg) or with the 5-HT reuptake inhibitor citalopram (30 mg/kg), however, failed to produce any effect alone or influence the response to MDMA despite blocking MDMA-induced cortical 5-HT loss. As MDMA also provokes the release of dopamine in the brain, and dopamine may lead to vasodilatation subsequent to dopamine D_1 receptor activation on cerebral microvessels, the effect of the dopamine D_1 receptor antagonist SCH 23390 (1 mg/kg) was also determined. While D_1 receptor antagonism provoked a decrease in cerebral perfusion in the visual and parietal association cortex, it failed to influence the changes in cortical perfusion obtained with MDMA indicating that dopamine D_1 receptors play a role in regulating blood flow in some brain regions but not MDMA-related perfusion changes in the frontal cortex. In conclusion although 5-HT depletion may play a role in mediating changes in cortical perfusion associated with MDMA administration, mechanisms independent of 5-HT such as direct drug action on, or 5-HT and dopamine D_1 receptor independent regulation of the cerebral microvasculature unit should also be considered.

Finally as repeated MDMA exposure leads to long-term 5-HT depletion, long-term changes in CBF and CBV were also assessed 8 weeks following a repeated regime of MDMA (5 and 10 mg/kg; i.p., twice daily for 4 days). Prior exposure to MDMA, having no effect alone, attenuated perfusion changes associated with acute MDMA (20 mg/kg) challenge. In addition, prior MDMA exposure was associated with a long-term reduction in cortical 5-HT concentrations. The results suggest that a functional deficit develops with prior exposure in relation to cerebrovascular tone and/or neurovascular coupling in response to acute challenge. The results have implications in relation to long-term deficits in the regulation of cerebral perfusion associated with prior MDMA exposure.

In conclusion this investigation illustrates the application of btASL MRI for determination of cerebral blood perfusion changes in response to MDMA administration in a rodent model and proposes that btASL MRI is a useful investigational tool with translational potential.