A comparison of MDMA (Ecstasy) and 3,4-methylenedioxymethcathinone (Methylone) in their acute behavioural effects and development of tolerance in rats

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A thesis submitted in partial fulfilment of the requirements for the Degree of Master of Science in Psychology

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Abbreviations

5-HT	Serotonin
ANOVA	Analysis of Variance
C	Celsius
cm	Centimetres
DMTS	Delayed matching to sample
EPM	Elevated plus maze
h	Hours
i.p.	Intraperitoneal
LDB	Light/Dark box
MDMA	3, 4-methylenedioxymethamphatemine
Methylone	3,4-methylenedioxymethcathinone
mg	Milligrams
mg/kg	Milligrams per kilogram
DA	Dopamine
DAT	Dopamine transporter
DN	Drug-naïve
DT	Drug-treated
NA	Noradrenaline
NAcc	Nucleus accumbens
NAT	Noradrenaline transporter
NOR	Novel object recognition
SEM	Standard error of the mean
SERT	Serotonin transporter

Abstract

Methylone (3,4-methylenedioxymethcathinone), the β-ketone analogue of the popular party drug MDMA (3,4-methylenedioxymethamphetamine, "ecstasy"), is a relatively new designer drug that is reported to have similar subjective effects and psychopharmacological properties to MDMA. However, unlike MDMA, little is known about the acute behavioural effects or the effects of repeated use of this drug. The goal of the current thesis was to investigate the behavioural effects of methylone and compare these to the effects of MDMA using an animal model. The second aim was to determine whether there was evidence of behavioural sensitisation or tolerance to methylone with repeated exposure. To achieve this, 108 male and female PVG/c hooded rats (6M and 6F per group) were administered various doses of MDMA or methylone (2.5, 5, 8, 12mg/kg), or saline vehicle (i.p.). The behavioural effects of these drugs were examined 20 m later, including horizontal locomotor activity, rearing behaviour, and central occupancy of an open field, anxiety behaviours in a light/dark box, and working memory in a novel object recognition task. The results showed that MDMA and methylone administration produce similar, but not identical, behaviours. Methylone was shown to produce greater psychostimulant effects, while MDMA produced more toxic effects. Female rats demonstrated greater psychostimulant effects than males, while males had higher rates of lethality. In order to assess the effects of repeated drug use, one week after binge-type drug administration of MDMA or methylone (5 mg/kg for 3 doses every 1h on 2 consecutive days), open field and light/dark box testing was repeated following a further 5 mg/kg challenge of drug. There was no evidence of locomotor sensitisation in the open field, although females showed sensitisation in rearing activity. These findings suggest that methylone may produce less toxic, but more stimulant, effects than MDMA. Methylone may therefore be a cocaine-MDMA mixed psychostimulant, both in a psychopharmacological and a behavioural sense.

1.1 Background

MDMA (3,4-methylenedioxymethamphetamine), known by the street name "ecstasy", is an illegal party drug that is known to produce a range of pleasant effects in users including euphoria, energy, empathy, and warmth towards others. Ever since being classified as an illegal substance people have been searching for non-scheduled alternatives with similar psychopharmacological effects (Bossong, Van Dijk, & Niesink, 2005). The use of novel synthetic psychoactive substances has therefore been increasing worldwide in recent years (Palamar, Martins, Su, & Ompad, 2015). For example, by 2013 the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) had reported the occurrence of over 200 new psychoactive substances in Europe, with new compounds being added every week (Iversen, White, & Treble, 2014). These drugs tend to be synthetic analogues of other illicit compounds, such as MDMA or N-substituted piperazines, and are often created to mimic these drugs while evading law enforcement. These substances are sold on the internet and smart shops under guises such as "bath salts" or "plant food", with a warning that they are not for human consumption, in order to conceal their use as drugs (P. S. Johnson & Johnson, 2014).

One of the more prevalent of these novel psychoactive substances is the synthetic cathinone "methylone" (2-methylamino-1-[3,4-methylenedioxyphenyl]propan-1-one, bk-MDMA), which is the β-ketone analogue of MDMA (Lopez-Arnau et al., 2013). Cathinone is a naturally occurring beta-ketoamphetamine analogue found in the leaves of the *Catha edulis* (Khat) plant. The synthetic cathinones are derivatives of this substance and are amongst the most common constituents of "bath salts"; a diverse group of designer drugs in the phenethylamine chemical class (Palamar, 2015). Since the subjective effects of methylone and other synthetic cathinones are said to be similar to other amphetamine derivatives, the use of these substances has been increasing rapidly worldwide as legal alternatives (Brunt et al., 2016;

Yin & Ho, 2012). While recreational doses enhance mood and increase alertness, higher doses have been associated with numerous adverse health outcomes (Lehner & Baumann, 2013). For example, in the US in 2011 there were over 20,000 emergency depatment visits and 6,137 poisonings from the use of “bath salts” (Palamar, Salomone, Vincenti, & Cleland, 2016).

Owing to the public health risks imposed, methylone and several related compounds were temporarily classified as Schedule I in the US by the DEA in October 2011. Methylone was extended into this schedule permanently in 2013 (Lehner & Baumann, 2013; Lopez-Arnau et al., 2013). In New Zealand, methylone is not specifically scheduled in the Misuse of Drugs Act 1975, however it is considered to be an amphetamine analogue, and is therefore a Class C prohibited substance.

While much is known about the acute and chronic effects of MDMA use, very little is known about the behavioural effects of methylone. This will be the topic of the current enquiry.

1.2 MDMA

MDMA is a substituted phenethylamine which is structurally similar to methamphetamine and mescaline. It was first synthesised and patented in 1912 by the pharmaceutical company Merck as a precursor in a new chemical pathway in the synthesis of a clotting agent “hydrastinine”, however basic toxicological and pharmacological testing was not conducted until years later (Freudenmann, Oxler, & Bernschneider-Reif, 2006). Interestingly, the first formal studies on toxicology and behavioural pharmacology in animals were conducted at the University of Michigan in 1953-54 by the US army, and were therefore classified until 1969 (Shulgin, 1986).

Some of the earliest reports of the psychopharmacological effects in humans come from research studies by Alexander Shulgin, finding that MDMA induces an easily controlled state

of altered consciousness (Shulgin, 1986). Soon after, a number of behavioural studies were undertaken which showed that MDMA acted as an analgesic and CNS stimulant in mice (Braun, Shulgin, & Braun, 1980). Toxicological studies in animals at substantially lethal doses found that MDMA caused a spectrum of behaviour including tremors, salivation, emesis, and death in a number of animals.

MDMA was classified as a Schedule 1 substance by the DEA in the US in 1985 on the grounds that it was a potential neurotoxin and due to the opinion that MDMA had no accepted medical use and high abuse potential (Cole & Sumnall, 2003a). In New Zealand, MDMA is currently classified as a Schedule 2 Class B controlled substance.

Despite being outlawed in the mid-1980s MDMA continues to maintain widespread popularity, particularly amongst young adults. In NZ, in a national household survey of people between 15 and 45 years old, it was found that the self-reported use of ecstasy in the last year had increased significantly from 1.5% in 1998 to 3.9% in 2006 (Wilkins & Sweetsur, 2008). In the US in 2008, 12.8% of young adults aged 18 to 25 years in a nationally representative sample reported lifetime MDMA use, with rates of use much higher in the subset of the population that attend raves or other electronic dance music (EDM) events (Palamar et al., 2016).

1.2.1 Psychopharmacology of MDMA.

Amphetamine derivatives, such as MDMA, act by enhancing release of central monoamine neurotransmitters (Cozzi, Sievert, Shulgin, Jacob, & Ruoho, 1999; Gudelsky & Yamamoto, 2008; Rothman & Baumann, 2003), and it has been extensively demonstrated that both serotonergic and dopaminergic mechanisms are responsible for the unique behavioural effects of MDMA (Gudelsky & Yamamoto, 2008).

The increased extracellular monoamine concentrations have been shown to occur through two distinct mechanisms (Cozzi et al., 1999). The first mechanism is through reduced uptake of released monoamines by inhibition of their specific transporters. There are transporter proteins expressed by serotonergic (SERT), dopaminergic (DAT) and noradrenergic (NAT) neurons whose function is to uptake released monoamine neurotransmitters from the synaptic cleft back into the neuron after release. This is the principle mechanism for inactivation of monoamine signalling (Rothman & Baumann, 2003). MDMA has been shown to potently block all three monoamine transporters causing a reduction in uptake after they are released and increased action of these neurotransmitters on their target receptors (Iravani, Asari, Patel, Wieczorek, & Kruk, 2000; Nagai, Nonaka, & Satoh Hisashi Kamimura, 2007; Steele, Nichols, & Yim, 1987). The second mechanism involves MDMA acting as a competing substrate at these transporter proteins where it enters the nerve ending via substrate specific carrier-mediated transport (Crespi, Mennini, & Gobbi, 1997). Once inside it has two effects which result in increased efflux of monoamine neurotransmitters. Firstly, it causes neurotransmitter release from intracellular storage vesicles resulting in an increase in cytoplasmic concentrations available for release. Secondly, it promotes neurotransmitter release through a process of transporter-mediated exchange by the monoamine transport proteins (Crespi et al., 1997; Rothman & Baumann, 2003; Rudnick & Wall, 1992; Sulzer et al., 1995). MDMA is therefore an indirect agonist of serotonin (5-HT), dopamine (DA), and noradrenaline (NA), inducing release of these monoamines from nerve terminals via transporter dependent mechanisms (Rothman & Baumann, 2003; Rudnick & Wall, 1992; Scearce-Levie, Viswanathan, & Hen, 1999).

MDMA is by far a more potent releaser of 5-HT than DA or NA (Schmidt & Kehne, 1990). It has been shown that the most characteristic acute effect of MDMA in animals is rapid release of 5-HT from presynaptic vesicles and serotonin reuptake inhibition (Crespi et al., 1997;

Iravani et al., 2000), with a dose-dependent increase in concentrations of 5-HT in the striatum, hippocampus, and cortex (Gough, Ali, Slikker, & Holson, 1991; Gudelsky & Nash, 1996). By 6 hours post administration there is a decline in the behavioural effects of MDMA as 5-HT efflux ceases, followed by a gradual recovery of 5-HT levels over the next 24 hours (Schmidt, Levin, & Lovenberg, 1987).

The effects of MDMA on the dopaminergic system are less pronounced, a property which separates MDMA greatly from other amphetamines, which have very potent effects on dopamine release (Gazzara, Takeda, Cho, & Howard, 1989; Spanos & Yamamoto, 1989). MDMA has been shown to stimulate the release of DA and block the reuptake of DA into brain synaptosomes (M. P. Johnson, Hoffman, & Nichols, 1986; M. P. Johnson, Huang, & Nichols, 1991; Schmidt et al., 1987; Spanos & Yamamoto, 1989; Steele et al., 1987). Activation of the 5-HT_{2A} receptor has been shown to increase dopamine synthesis and release, suggesting that DA release may be, at least partly, due to 5-HT release (Gudelsky, Yamamoto, & Nash, 1994). It is likely that this DA release is related to the mild euphoria and rewarding properties of MDMA (Gudelsky & Yamamoto, 2008).

Further experiments found similar releasing effects on NA (Rothman et al., 2001). Increased levels of NA are not known to correlate with the intoxicant effects of the drug, but it is likely to contribute to the sympathomimetic effects via activation of adrenergic receptors, resulting in potentially dangerous cardiovascular side-effects, such as increased blood pressure (Iversen et al., 2014; Vollenweider, Liechti, Gamma, Greer, & Geyer, 2002). Finally, MDMA has been shown to release Ach (Acquas et al., 2001), although this is to a much lesser degree than 5-HT release. This is likely due to direct activation of histamine 1 (H1) receptors (Vollenweider et al., 2002).

1.2.1 Acute behavioural effects of MDMA in human users.

Subjective effects.

The chemical structure of MDMA is similar to both amphetamine stimulants and hallucinogens, however the behavioural pharmacology of this compound is distinct from both of these broad drug classes (Fantegrossi, 2008). Human users of MDMA report subjective effects including euphoria, altered time perception, increased alertness, luminescence of objects, decreased hostility, and powerful feelings of closeness and empathy towards others. Negative effects included nausea, insomnia, bruxism, dry mouth, diaphoresis, palpitations, tremor, and increased body temperature (Palenicek, Votava, Bubenikova, & Horacek, 2005; M. Tancer & Johanson, 2003). The term 'entactogen' has been proposed to describe the effects of MDMA, literally meaning "producing a touching within", which refers to the drugs ability to allow therapists and patients to access and deal with repressed painful emotional issues (Cole & Sumnall, 2003a; Nichols, 1986). Although the hallucinogenic properties of MDMA are considered to be weak, some users have reported hallucinogenic effects at higher doses (Solowij, Hall, & Lee, 1992).

Many of the anecdotal reports of the subjective effects of MDMA are hindered by the fact that 'ecstasy' tablets often do not solely contain the active compound MDMA, but may include a range of other psychoactive compounds, such as amphetamine, ketamine, or ephedrine (Cole and Sumnall, 2003). Subjective effects of MDMA have therefore been extensively investigated in controlled laboratory settings using various scales, including the POMS (Profile Of Mood States), VAS (Visual Analog Scale), HRS (Hallucinogen Rating Scale), and SDEQ (Subjective Drug Effects Questionnaire); e.g. (Cami et al., 2000; Harris, Baggott, Mendelson, Mendelson, & Jones, 2002; Kuypers & Ramaekers, 2005; M. E. Tancer & Johanson, 2001).

Reported effects have been recorded to peak at 75 to 120 minutes after consumption, last two to twelve hours, and include mostly positive effects on mood ratings with a state of enhanced mood and well-being (Baylen & Rosenberg, 2006; Vollenweider, Gamma, Liechti, & Huber, 1998). Positive effects include excitement, clearer thinking, affection or closeness to others, confidence, euphoria, elation, vigour, peacefulness, and relaxation. In contrast, negative emotional effects appear to have lower prevalence rates and tend to be associated with higher doses. These acute effects include autonomic hyperactivity, anxiety, restlessness, and confusion (Baylen & Rosenberg, 2006; de Sousa Fernandes Perna et al., 2014; Downing, 1986; Harris et al., 2002; M. E. Tancer & Johanson, 2001; Vollenweider et al., 1998). Somatic effects include teeth clenching, temperature changes, nausea, reduced appetite, tremors, mydriasis, and sweating (Baylen & Rosenberg, 2006; Downing, 1986; Vollenweider et al., 1998). Some of the somatic effects of acute intoxication were present 24 hours post ingestion, such as restlessness, suppressed appetite, bruxism, and difficulty concentrating, while new after effects were evident in some subjects, including lack of energy and insomnia (Vollenweider et al., 1998). Women tended to show stronger responses to MDMA than men, with significantly higher ratings for positive mood, anxiety, and somatic effects (Liechti, Gamma, & Vollenweider, 2001).

Neurocognitive effects.

Acute intoxication with MDMA has been shown to cause impairment in memory. This has been demonstrated in a number of studies on human participants using single MDMA doses, with MDMA induced impairment of immediate and delayed recall for both verbal and spatial information (Kuypers, de la Torre, Farre, Pujadas, & Ramaekers, 2013; Kuypers & Ramaekers, 2005, 2007; Stough et al., 2012; van Wel et al., 2011). Kuypers and Ramaekers (2005) tested the effect of a single moderate dose of MDMA (75 mg) on memory functioning in MDMA users in a double blind placebo-controlled crossover design. The neurocognitive assessments included a verbal word learning task with immediate recall (learning/working

memory) and delayed recognition (long term/episodic memory), a syntactic reasoning task (working memory and speed), and a digit symbol substitution task (short term memory). They found that MDMA impaired working memory during intoxication as assessed by the number of words immediately recalled in the word learning task, as well as an impairment in delayed recall after 30 minutes, with no residual deficit in memory function after a 24 hour withdrawal phase. This suggests that acute intoxication of MDMA causes an impairment in working memory, but does not produce a permanent memory deficit from a single dose. Impaired working memory during acute MDMA intoxication has also been reported using other cognitive performance tasks (de Sousa Fernandes Perna et al., 2014; Stough et al., 2012; van Wel et al., 2011). In addition, an impairment of spatial memory for location has been demonstrated in acute MDMA intoxication using a spatial memory task (Kuypers & Ramaekers, 2007; van Wel et al., 2011).

Taken together, these results suggest that acute MDMA intoxication may produce reversible memory impairments in working and spatial memory. However, studies investigating the acute effects of MDMA on working memory and learning tasks have in general failed to test for non-mnemonic causes of memory impairment, such as increased distraction from non-task related events or stimuli, poorer concentration, or impaired psychomotor performance. Indeed, participants in such studies have previously suggested that impairments in their performance may have been due to more general problems in attending to the relevant task (Kay, Harper, & Hunt, 2010; Parrott & Lasky, 1998). Therefore, whether there is an impairment of memory storage or a more general impairment in cognitive processes in humans remains unclear, which places greater importance in the outcomes of animal research.

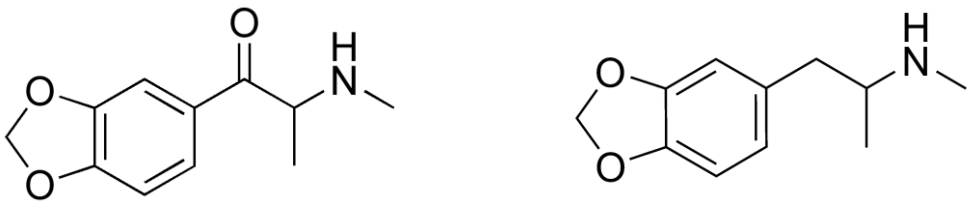
1.3 Methylone

Methylone was first synthesised and named by Alexander Shulgin while researching the effects of betaketone modification of amphetamines. However it was not until years later, in 2004, that it began to appear in the Netherlands as a new designer drug called 'Explosion' (Bossong et al., 2005). It was advertised as a vanilla-scented room odouriser in plastic tubes containing 5mL of liquid, and contained the instructions 'Do not ingest' in order to circumvent Dutch regulations for psychoactive substances (Bossong et al., 2005). In New Zealand, methylone was sold legally as an MDMA substitute under the brand name "Ease". It was withdrawn from public supply in April 2006 due to the opinion that it was an amphetamine analogue, and is therefore considered a Class C substance.

Over the past few years methylone has become one of the main constituents of "bath salts" and one of the most frequently abused synthetic cathinones in the US (German, Fleckenstein, & Hanson, 2014). These substances are most frequently insufflated, although many users take them orally in tablet form or dissolved in beverages, rectally, or parenterally (German et al., 2014; Karila, Billieux, Benyamina, Lancon, & Cottencin, 2016).

1.3.1 Psychopharmacology of methylone.

Chemically, methylone is the β-ketone analogue of MDMA, differing by the addition of a ketone oxygen at the benzylic position of the molecule (*Figure 1*).



Methylone **MDMA**

Figure 1.1. Chemical structure of methylone (3,4-methylenedioxymethcathinone) and MDMA (3,4-methylenedioxymethamphetamine).

The differences in the behavioural effects of psychostimulants have been shown to reflect the relative changes in extracellular monoamine concentrations (Iversen et al., 2014). This is also likely to be an important mechanism for the behavioural effects of the synthetic cathinones (Gatch, Taylor, & Forster, 2013). Since methylone has been reported as having similar subjective effects to MDMA, and shares a similar chemical structure, these two substances should produce a similar pharmacological profile at monoamine plasma membrane transporters. Indeed, several studies examining the neurobiological effects of methylone support this rationale.

Methylone is a potent uptake inhibitor of all three monoamines as well as a substrate for all three monoamine transporters. Simmler et al. (2013) determined the potencies of several cathinones to inhibit DA, NA, and 5-HT transport in HEK 293 cells expressing human monoamine transporters *in vitro*, as well as their ability to promote DAT and SERT-mediated DA and 5-HT efflux. They found that both MDMA and methylone blocked all three monoamine transporters. MDMA blocked SERT significantly more than DAT, whereas methylone was similar to cocaine and methamphetamine with higher DAT selectivity than SERT but with much less potency (Simmler et al., 2013). In a study examining inhibition of monoamine uptake transporters in human platelet cells, Cozzi et al. (1999) found that

methylone was as potent as MDMA at inhibiting DA and NA reuptake, but three-fold less potent than MDMA at inhibiting serotonin reuptake *in vitro* (Cozzi et al., 1999). This was consistent with other research using rat brain synaptosomes (Baumann et al., 2012; Nagai et al., 2007). NAT inhibition was more potent than DAT and SERT inhibition for both MDMA and methylone (Nagai et al., 2007).

In addition to blocking reuptake, methylone functions as a substrate for monoamine transporters *in vitro*, stimulating the release of DA and 5-HT by reversal of the normal transporter efflux (Baumann et al., 2012; Simmler et al., 2013). Methylone was similar to MDMA in its substrate activity, with greater efflux of 5-HT than DA, although the potency of methylone was lower. Methylone has been shown to be half as effective as MDMA at increasing DA release and one-third as effective as MDMA at increasing 5-HT release (Nagai et al., 2007; Sogawa et al., 2011). The addition of the β-ketone to MDMA appears, therefore, to increase the compounds selectivity for the DAT than SERT and reduce its overall potency. The relative effects of a drug on the DAT and SERT are useful to predict the drugs characteristics *in vivo* (Simmler et al., 2013).

The *in vivo* neurochemical actions of methylone produced elevations in DA and 5-HT which were quantitatively similar to the effects of MDMA with preferential effect on 5-HT, but again with less potency. This adds support to the idea that methylone is closer in neurochemical effects to MDMA than cocaine or amphetamines (Baumann et al., 2012), although its ability to release 5-HT is diminished relative to DA. Because 5-HT release dampens the stimulant effects of amphetamine-like drugs, we would expect that methylone would have more stimulant-like effects when compared to MDMA (Baumann et al., 2012; Simmler et al., 2013). Methylone may also be associated with increased risk of addiction than MDMA because of its higher relative action on the DA system. Given this profile of effects on

monoamine transporters, it has been suggested that this compound is a cocaine-MDMA-mixed cathinone, and may therefore demonstrate behavioural effects similar to both MDMA and cocaine (Simmler et al., 2013).

1.3.2 Acute behavioural effects of methylone in human users.

While the number of studies on the effects of methylone is humans scant within the scientific literature, anecdotal reports suggest that it shares similar subjective effects with MDMA, as expected from the pharmacology of this drug (Bossong et al., 2005). Human users of methylone have reported via consumer websites that methylone provides entactogenic effects but with a calmer euphoria and milder stimulation than that experienced from MDMA (Shimizu et al., 2007). Shulgin wrote 'methylone has almost the same potency as MDMA, but it does not produce the same effects. It has almost antidepressant action, pleasant and positive, but not the unique magic of MDMA' (Bossong et al., 2005).

Despite its widespread use, no studies currently exist that have characterised the subjective or neurocognitive effects of methylone in humans in a controlled setting.

1.4 Animal Studies

1.4.1 Acute behavioural effects of MDMA.

Numerous studies exist investigating the acute and long-term behavioural effects of MDMA in animals (Cole & Sumnall, 2003b). These studies have largely focussed on locomotor activity, anxiety, social behaviour, exploratory behaviour, memory, and reward and reinforcement. In animals acute administration of MDMA produces neural excitability with hyperthermia, hyperactivity, low body posture, salivation, piloerection, ataxia, and mydriasis (Spanos & Yamamoto, 1989). The current study will examine the effect of MDMA

administration in the rat on several behavioural outcomes including locomotor activity, exploratory behaviours, anxiety, and memory. The current literature regarding the acute effects of MDMA on these behaviours is summarised below.

Locomotor activity.

Animal models of locomotor behaviour provide us with an understanding of the complex interactions between neurochemical systems and the behavioural effects of drugs of abuse, particularly the stimulants and hallucinogens (Risbrough et al., 2006). The characterisation and quantification of locomotor paths and investigatory behaviours has demonstrated unique behavioural patterns for the psychostimulant drugs, leading to a greater understanding of their pharmacological mechanisms (Paulus & Geyer, 1992; Risbrough et al., 2006).

Administration of MDMA has been shown to dose-dependently (0 to 20 mg/kg) increase horizontal locomotor activity in the open field test in rats (Callaway, Johnson, Gold, Nichols, & Geyer, 1991; Callaway, Wing, & Geyer, 1990; Herin, Liu, Ullrich, Rice, & Cunningham, 2005; Kehne et al., 1996; Spanos & Yamamoto, 1989), with females demonstrating a higher sensitivity to the stimulating effects of MDMA than males (Palenicek et al., 2005). While the interplay of neurotransmitter systems and receptors involved is not yet fully understood, it has been consistently shown that MDMA induces locomotor hyperactivity through indirect actions on both DA and 5-HT systems (Bubar, Pack, Frankel, & Cunningham, 2004; Callaway et al., 1990; Gold, Hubner, & Koob, 1989; McCreary, Bankson, & Cunningham, 1999).

The locomotor activating effect of MDMA is distinct from other amphetamines since the initiating event seems to be activation of serotonergic receptors, particularly 5-HT_{1B} (Bankson & Cunningham, 2002; McCreary et al., 1999; Rempel, Callaway, & Geyer, 1993).

This is based on the observation that 5-HT_{1B} receptor agonists produce a behavioural profile similar to MDMA, with almost identical spatial patterns of locomotion (Bankson & Cunningham, 2002; Rempel et al., 1993). In addition, the highly selective 5-HT_{1B} antagonist GR127935 significantly and dose-dependently attenuated the locomotor stimulatory effects of MDMA in both Sprague-Dawley and Wistar rats back to the level of controls (McCreary et al., 1999; Steed, Jones, & McCreary, 2011). At higher doses MDMA-stimulated locomotion is augmented by 5-HT_{2A} receptors (Herin et al., 2005; Kehne et al., 1996), while 5-HT_{2C} receptors have a large inhibitory role on locomotor activity (Fletcher, Sinyard, & Higgins, 2006; Steed et al., 2011). This activation of serotonergic receptors has been shown to exert its behavioural effect on locomotion by indirectly increasing DA release, particularly in mesolimbic pathway which projects to the nucleus accumbens (NAc) (Bubar et al., 2004; Callaway et al., 1991; Gold et al., 1989; McCreary et al., 1999). In support of this, the selective serotonin reuptake inhibitor fluoxetine has been shown to attenuate MDMA induced hyperactivity, with corresponding reduction in the efflux of DA in the striatum (Callaway et al., 1990; Gudelsky & Yamamoto, 2008). In addition, dopamine antagonists dose-dependently attenuated MDMA induced hyperactivity, indicating that activation DA receptors is integral to the locomotor stimulating effects of this drug (Bubar et al., 2004; Kehne et al., 1996). A rich interplay of 5-HT and DA systems must therefore underlie the locomotor stimulatory effects of MDMA, whereby activation of both 5-HT and DA receptors are vital to this response.

While both MDMA and amphetamine increase locomotor activity in the open field in rats, the spatial pattern of movement induced by each drug has been shown to differ (Martinez-Price & Geyer, 2002). The type of locomotion induced by MDMA is characterised by a pattern of persistent forward locomotion predominantly around the perimeter of the chamber with avoidance of the centre, interrupted by occasional changes in direction (*Figure 1.2*) (Bankson & Cunningham, 2002; Callaway et al., 1991; Martinez-Price & Geyer, 2002; McCreary et al.,

1999; Rempel et al., 1993), although increased central activity has also been observed (Kindlundh-Hogberg, Schioth, & Svenningsson, 2007; McCreary et al., 1999). Amphetamines, on the other hand, induce non-repetitive patterns of behaviour with frequent changing of direction that is distributed throughout all regions of the chamber including relatively long periods in the centre (Callaway et al., 1990; Geyer, Russo, Segal, & Kuczenski, 1987; Gold & Koob, 1989; Rempel et al., 1993). Therefore, while both MDMA and amphetamines may increase quantity of locomotor activity, the behavioural patterns produced by these two drugs seem to be characteristically distinct (Callaway et al., 1990).

Decrease in central activity following MDMA exposure is likely related to augmentation of the serotonergic system, since this behaviour is not seen in amphetamine treated rats. In keeping with this, Rempel et al. (1993) found that a 5-$HT_{1A/1B}$ receptor agonist increased locomotor activity that was preferentially located in the periphery of the chamber similar to MDMA, supporting a role for 5-HT in the change in spatial pattern (Rempel et al., 1993).

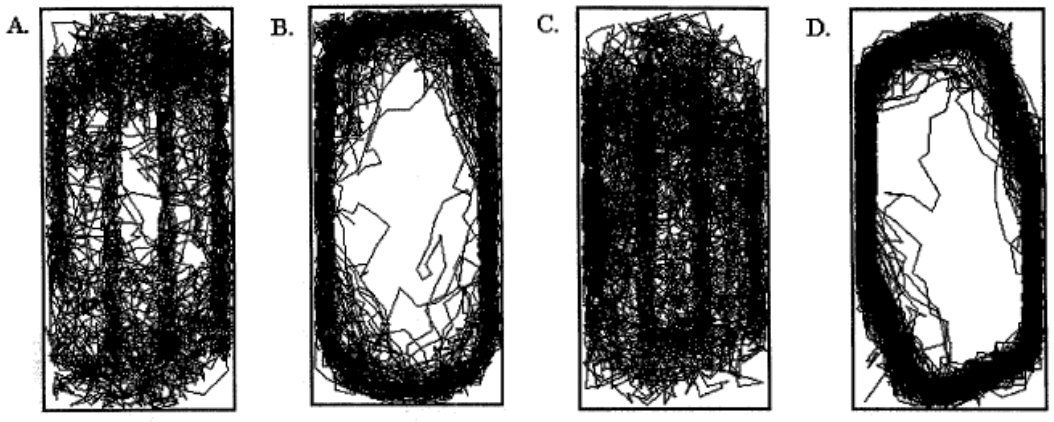


*Figure 1.2. The locomotor paths of rats injected with **A**. Saline, **B.** MDMA, **C**. amphetamine, or **D**. a 5-HT1A/1B receptor agonist. Note that the locomotor activity after administration of MDMA or 5-HT1A/1B agonist appears to be mostly confined to the periphery of the chamber. Adapted from Martinez-Price et al. (2002).*

Exploratory behaviour.

Other behaviours that have been widely examined in open field tests with psychostimulant drugs are exploratory behaviours, such as rearing and nose holepokes. Rearing behaviour consists of animals standing on their hind legs in an upright posture and is considered to be a risk assessment behaviour, an orienting response, a means of scanning the environment, or a marker of environmental novelty (Ennaceur, 2014). Holepokes involve the animal poking its head into a hole in the floor of the testing apparatus, and is considered to be a measure of directed exploration, or neophilia (Brown & Nemes, 2008). A reduction in rearing behaviour or holepokes is generally thought to represent increased levels of anxiety, although it has also been considered to relate to general locomotor or exploratory activity (Thiel, Muller, Huston, & Schwarting, 1999).

There is mixed evidence regarding the effect of MDMA on these exploratory behaviours in rats and mice (summarised in Table 1.1). Most studies have reported a significant reduction in rearing and holepoke activity in both rats and mice at doses greater than 3 mg/kg, while at lower doses there may be a paradoxical increase in rearing activity. Callaway et al. (1990) found in a study using male Sprague-Dawley rats that both rearings and holepokes were significantly reduced following doses of MDMA of 1.0 mg/kg or higher (Callaway et al., 1990). In male CD rats, Kehne et al. (1996) determined that MDMA only suppressed rearing at doses of 20 mg/kg or higher, indicating that there may be strain dependent differences in sensitivity to MDMA (Kehne et al., 1996). In mice, reductions in rearing and holepoke activity have been consistently reported in doses greater than 3.3 mg/kg (Ferraz-de-Paula et al., 2011; Maldonado & Navarro, 2000; Scearce-Levie et al., 1999). Alternatively, other studies using Sprague-Dawley rats have found either no effect or an increase in rearing activity at doses of

3mg/kg (Bankson & Cunningham, 2002; Bubar et al., 2004; McCreary et al., 1999). Taken together these studies suggest that lower doses of MDMA may cause an increase in rearings while higher doses may suppress rearings. This paradoxical effect is supported by Palenicek et al. (2005) who found that 2.5 mg/kg increased, while 10mg/kg decreased, rearing activity in male Wistar rats.

Contrary to these findings, Hernin et al. (2005) reported an increase in rearing activity using a similar paradigm in male Sprague-Dawley rats in doses up to 12 mg/kg, with a maximal effect seen at 8mg/kg, suggesting an inverse-U-shaped dose-response curve (Herin et al., 2005). A possible explanation for this discrepancy in findings may relate to the methodology employed between studies; specifically, whether the rats were habituated to the testing chamber prior to testing. Introducing the animals to the testing arena prior to experimentation is expected to produce a reduction in exploration as the animal becomes familiar with the environment (Brown & Nemes, 2008; Hughes, 2007a). Therefore, habituation to the testing arena would serve to reduce rearing activity due to a lower neophilic exploratory drive. In the study by Herin et al. (2005) the experimenters habituated the rats for three hours per day for the three days prior to testing. The effect of habituation in this study is demonstrated by low amounts of rearing activity in the control animals, with less than 50 rearings on average for the 90 minute session. In contrast, Callaway et al. (1990) had no habituation sessions with their saline control rats performing approximately 130 rearings on average over a 60 minute session. This can be interpreted as a higher drive to explore the novel environment. On the other hand, habituation would be predicted to decrease stress-induced neophobia to a novel environment in MDMA exposed rats (Belzung & Griebel, 2001; Pare, Tejani-Butt, & Kluczynski, 2001). The fact that MDMA treated rats in the Callaway et al. (1990) study performed more rearings in the second 30 minutes compared to the first 30 minutes may be related to reduced anxiety as these animals became habituated to the testing arena. Indeed, the behaviourally suppressive effects of other

hallucinogenic 5-HT_2 agonists on investigatory behaviours have been shown to disappear in a familiar environment (Mittman & Geyer, 1989; Wing, Tapson, & Geyer, 1990).

It seems, therefore, that the effect of habituation to the apparatus is to decrease rearing in saline control animals due to reduction in neophilia, while increasing the exploratory activity in MDMA treated rats due to reduction in neophobia. This hypothesis is supported by another experiment by Camarasa et al. (2008) who examined whether memantine (a nicotinic and NMDA receptor antagonist) would reverse memory impairments caused by acute MDMA administration in rats. In this experiment they habituated rats for six hours for the two days prior to testing, which they found almost eliminated rearing behaviour in the saline control rats, while MDMA treated rats (10 mg/kg) were found to have a significantly higher amount of rearing activity (Camarasa, Marimon, Rodrigo, Escubedo, & Pubill, 2008). Thus, habituation may, at least partly, explain the discrepancies between studies regarding the effect of MDMA on rearing activity.

To conclude, MDMA may have an activating effect on exploratory behaviour at low doses, with an inhibitory effect at higher doses. Suppression of rearing activity may be a result of the anxiogenic effects of MDMA combined with the aversive effects of a novel environment. Alternatively, increased rearing activity in habituated rats may be due to reduced neophobia coupled with psychomotor stimulation. Future research addressing the effect of habituation on the acute behavioural effects of MDMA and other psychostimulants is warranted.

Table 1.1. Summary of animal studies examining the effects of MDMA on exploratory activity.

Study	Animals used	Dose	Behavioural test	Outcome
Callaway et al. (1990)	Male Sprague-Dawley rats	0.3, 1.0, 3.0, 10.0mg/kg SC	Rearings and holepokes	Dose-depdendently reduced rearing and holepokes at doses at 1mg/kg or higher
Scearce-Levie at al. (1999)	Male 129/Sv mice	3.3, 10, 30mg/kg IP	Rearings and holepokes	All doses eliminated rearing and holepoke activity
Kehne et al. (1996)	Male CD rats	1, 2, 3, 10, 20, 40mg/kg IP	Rearings	Higher doses (20-40mg/kg) significantly reduced rearing, no effect for doses 10mg/kg or less
Ferraz-De-Paula et al. (2011)	Male BALB/c mice	0.2, 1, 5, 8, 10, 20mg/kg IP	Rearings	Reduced rearing activity with 5mg/kg or higher doses, no significant effect at low doses
Maldonado & Navarro (2000)	Male OF.1 mice	1, 8, 15mg/kg IP	Rearings	Significant reduction in rearing at 8 and 15mg/kg
McCreary et al. (1999)	Male Sprague-Dawley rats	3mg/kg SC	Rearings	Inconsistent results
O'Loinsigh et al. (2001)	Male Wistar rats	20mg/kg IP	Rearings	Significant reduction in rearings
Bankson & Cunningham (2002)	Male Sprague-Dawley rats	3mg/kg SC	Rearings	Inconsistent results
Palenicek et al. (2005)	Male and Female Wistar rats	2.5mg/kg and 10mg/kg SC	Rearings	2.5mg/kg increased rearing while 10mg/kg decreased rearing activity
Bubar et al. (2004)	Male Sprague-Dawley rats	3mg/kg SC	Rearings	Significantly increased rearing
Herin et al. (2005)	Male Sprague-Dawley rats	2, 3, 4, 8, 12mg/kg SC	Rearings	Inverted-U-shaped response curve with maximal increase in rearings seen at 8mg/kg

Anxiety.

Anxiety is a negative emotional state associated with the perception of an ambiguous or potential threat (Ennaceur, 2014). Behavioural animal studies on MDMA and anxiety have produced mixed results and seem to demonstrate both anxiolytic and anxiogenic effects (Ferraz-de-Paula et al., 2011) (summarised in Table 1.2). Most studies have demonstrated an anxiogenic effect for MDMA in low to medium doses in a variety of behavioural tests. Doses of MDMA up to 10mg/kg have been shown to increase anxiety-like behaviours in the elevated plus maze (EPM) in mice and rats, with decreased total and open arm entries and markedly increased closed arm entries (Ho, Pawlak, Guo, & Schwarting, 2004; Lin, Burden, Christie, & Johnston, 1998; Morley & McGregor, 2000; Navarro & Maldonado, 2002). Morley & McGregor (2000) studied the effects of low doses of MDMA on male Wistar rats in a range of behavioural tests of anxiety including the EPM, a social interaction test, odour avoidance test, and footshock-induced ultrasonic vocalisations. They found that doses up to 5 mg/kg produced anxiogenic behaviour in most of these tests, with the exception of the social interaction test where there was a decrease in aggressive behaviour and increased duration of social interaction (Morley & McGregor, 2000). In the emergence test, emergence latency and increased hide time has been shown to be increased following low dose MDMA exposure in rats, again indicating an anxiogenic response (Jones, Brennan, Colussi-Mas, & Schenk, 2010; Morley, Arnold, & McGregor, 2005).

Higher doses of MDMA have been reported to produce different results on anxiety depending on the test employed. MDMA at doses of 10 to 20 mg/kg have been reported by several studies as producing anxiolytic behaviour including increased time in the centre squares of the open field and increased entries and time spent in the open arms of the EPM, with some authors suggesting that MDMA may have anxiogenic effects at low doses and anxiolytic effects at high doses (Ferraz-de-Paula et al., 2011; Ho et al., 2004; Lin et al., 1998; Palenicek et al.,

2005). However, in a predator odour test, Ferraz-de-Paula et al. (2011) found that 10 mg/kg MDMA increased the amount of time mice spent in the home chamber and decreased the number of risk assessments, suggesting increased levels of anxiety. The authors suggest that the apparent anxiolytic effect seen in the open field and EPM in these studies may have been due to MDMA altered locomotor activity rather than an effect on levels of anxiety per se. In support of this argument, Maldonado & Navarro (2001) found that 8 and 15 mg/kg MDMA resulted in a reduction in social investigation and an increase in avoidance and defence behaviours in a social interaction test in mice, which may represent anxiogenic behaviour. However, at these same doses there was no significant difference between MDMA and control rats in time spent in the light side of a light/dark box, although there was a significant reduction in transitions and rearing behaviour indicating a reduction in exploratory activity with no increased aversion to the bright light (Maldonado & Navarro, 2001).

Taken together these results suggest that MDMA may have both anxiolytic and anxiogenic effects depending on the test situation, dose, and type of animal employed (Ferraz-de-Paula et al., 2011; Morley & McGregor, 2000). MDMA tends to produce an anxiogenic response at low doses in most of these tests, while producing an anxiolytic effect in social interactions, suggesting that the prosocial effects may be mediated by different neural pathways than the effect on situational, or ‘state’, anxiety.

Table 1.2. Summary of animal studies examining the effect of MDMA on anxiety

Study	Animals used	Dose	Behavioural test	Outcome
Lin et al. (1998)	Male QS mice	1, 4, 12, 20mg/kg IP	EPM	4mg/kg decreased open arm entries and increased enclosed entries. 12mg/kg no effect on EPM. 20mg/kg increased time spent in the open arm.
Morley & McGregor (2000)	Male Wistar rats	1.25-5mg/kg IP	EPM, social interaction, cat odour avoidance, footshock ultrasonic vocalisations	Increased anxiety-like behaviours in EPM at all doses. 5mg/kg reduced time spent in proximity to cat odour stimulus and reduced footshock-induced ultrasonic vocalisations. Reduced aggressive behaviours and increased duration of social interaction.
Maldonado & Navarro (2000)	Male OF1 mice	1, 8, 15mg/kg IP	LDB	8 and 15mg/kg produced less transitions. No effect on time spent in light side of the box.
Maldonado & Navarro (2001)	Male OF1 mice	1, 8, 15mg/kg IP	Social interaction	8 and 15mg/kg produce decrease in aggression, reduced social investigation, and increase in avoidance and defence behaviours
Navarro & Maldonado (2002)	Male OF1 mice	1, 8, 15mg/kg IP	EPM	8mg/kg reduced time spent in open arms and increased number of entries in the closed arms. 15mg/kg had no effect on anxiety behaviours.
Ho et al. (2004)	Male Wistar rats	7.5 & 15mg/kg IP	EPM	7.5mg/kg increased latencies to enter open arms, less time in open arms, and more total arm entries, 15mg/kg produced increased open arm time.
Palenicek et al. (2005)	Male and Female Wistar rats	2.5 & 10mg/kg SC	EPM	10mg/kg produced increased time in the open arms.

Morley et al. (2005)	Male Wistar rats	5mg/kg IP	Social interaction, LDB	Increased social interaction including adjacent lying and approach behaviours. Increased hide time and emergence latency in emergence test.
Jones et al. (2010)	Male Sprague-Dawley rats	1 & 3.3mg/kg IP	Emergence latency	3.3mg/kg MDMA significantly increased emergence latency
Ferraz-De-Paula et al. (2011)	Male BALB/c mice	10mg/kg IP	EPM, predator odour test	In the EPM increased entries and time spent in the open arms.In a predator odour test increased time in the home chamber and decreased number of risk assessments.

Memory.

Surprisingly few studies have been conducted looking at the acute effects of MDMA on memory and learning, with most studies focussing on the long-term sequelae of repeated MDMA administration (Kay et al., 2010). Numerous studies exist examining the chronic effects of MDMA on learning and memory, in both humans and animals. Some of these reports suggest that there is lasting neurocognitive deficit in recreational human users of MDMA, although this is inconclusive due to multiple confounders including poly drug use and comorbid mental health problems (Moratalla et al., 2015; Schenk, Harper, & Do, 2011). MDMA administration in animals has not produced a clear set of cognitive changes (Able, Gudelsky, Vorhees, & Williams, 2006).

MDMA has been found to decrease accuracy and response rates in the delayed matching or non-matching to sample (DMTS or DNMTS) tasks in rats and pigeons after acute administration, indicating an impairment in working memory (Harper, Hunt, & Schenk, 2006; Harper, Wisnewski, Hunt, & Schenk, 2005; LeSage, Clark, & Poling, 1993; Marston, Reid, Lawrence, Olverman, & Butcher, 1999). Conversely, no significant impairment in this task was found in rhesus monkeys administered low-dose MDMA (up to 1 mg/kg) indicating that these deficits in working memory may only be apparent at higher doses (Frederick, Gillam, Allen, & Paule, 1995). However, it has been suggested that the overall impairment in the DMTS task may be due to an increased tendency to repeat the choice response made in the previous trial, known as proactive interference, rather than an impairment in working memory itself (Harper et al., 2006). This implies that rats either have trouble distinguishing between events that occurred in previous trials and the current one, or there is perseveration of responses indicating a lack of behavioural flexibility (Harper, 2013). In accordance with this, an increase in the time delay between trials was found to attenuate the MDMA induced impairment in this task, presumably by allowing rats to more easily discriminate current from previous-trial events,

indicating that working memory itself was not affected (Harper et al., 2006). Therefore, rats may remember episodic events within each trial, but they are impaired with respect to what they are supposed to do with the information they have, indicating impaired procedural, or reference, memory (Kay, 2010). Indeed, MDMA has been shown to preferentially disrupt reference memory processes in the eight-arm radial maze task in rats indicating that this drug impairs strategies for solving memory tasks which may, at least partly, explain apparent impairments in working memory seen in previous studies (Braida, Pozzi, Cavallini, & Sala, 2002; Kay et al., 2010).

From these studies it seems that MDMA may disrupt reference memory function while preserving working or short term memory. Alternatively, MDMA may disrupt both working and reference memory. More research on the acute effects of MDMA on memory are necessary using a variety of memory tasks in order to further clarify these memory deficits. The current paper will use a novel object recognition task, a test that has been shown to evaluate working memory, to this end.

1.4.2 Acute behavioural effects of methylone.

While there have been a number of studies looking at the pharmacology of methylone and new designer cathinones, relatively few studies have been conducted on the behavioural effects of methylone. Overall, the behavioural profile of methylone in animal studies has been shown to closely resemble that of MDMA (Gregg & Rawls, 2014). This is supported by the observation that methylone completely substitutes for MDMA in tests of stimulus generalisation in MDMA trained rats, with methylone (ED_{50}=1.6 mg/kg) being about half as potent as MDMA (ED_{50}=0.76 mg/kg) (Dal Cason, Young, & Glennon, 1997).

Consistent with the effects of psychostimulant drugs, several studies have found dose-dependent increases in locomotor activity following injections of methylone (3 to 30 mg/kg) in both mice and rats (Gatch et al., 2013; Lopez-Arnau et al., 2013; Lopez-Arnau, Martinez-Clemente, Pubill, Escubedo, & Camarasa, 2012; Marusich, Grant, Blough, & Wiley, 2012). In these studies the onset of action was rapid, with maximal stimulant effects between 0 and 30 minutes post-administration, and lasted up to 4h post-administration. An ED_{50} of 1.48 mg/kg was calculated for male Swiss-Webster mice (Gatch et al., 2013). The increase in psychomotor activity was inhibited following haloperidol or ketanserin pre-treatment, suggesting involvement of DA and 5-HT systems, consistent with MDMA (Lopez-Arnau et al., 2012). There has also been shown to be a significant increase in rearing and forepaw treading following methylone administration in rats (Baumann et al., 2012).

To date, no studies have directly compared the behavioural effects of methylone with MDMA in rats. While several studies have demonstrated increased locomotor effects after acute methylone administration, there appears to be no studies that have looked at the acute effects on memory and anxiety.

1.5 Effect of Repeated Drug Exposure

1.5.1 Effect of repeated exposure to MDMA.

Human users of ecstasy have reported diminished subjective effects with repeated exposure, leading to increasing amounts of MDMA being consumed (Parrott, 2005). This has been attributed to the reduced 5-HT neurotransmission that temporarily occurs following repeated MDMA exposure, which may last for months after abstinence (Green, Mechan, Elliott, O'Shea, & Colado, 2003; Jones et al., 2010; Schenk & Bradbury, 2015). While there is

a widespread reduction in brain 5-HT following repeated high dose MDMA exposure, studies in rats have shown a simultaneous increase in DA neurotransmission in the NAc (Kalivas, Duffy, & White, 1998; Mayerhofer, Kovar, & Schmidt, 2001). Thus, repeated MDMA administration may lead to a behavioural tolerance to the serotonergic effects with concomitant sensitisation to dopaminergic effects (Schenk, 2011). Indeed both tolerance to, and sensitisation to, many of the behavioural effects of MDMA have been observed following chronic exposure (Jones et al., 2010).

Human MDMA use also often involves repeated drug administration within a single session, known as 'binge dosing', in order to increase the subjective effects of the drug over a longer period of time (Docherty & Green, 2010). A number of studies have examined the effect of repeated exposure of MDMA using a variety of dosing paradigms. In rodents, a common research approach to model repeated exposure is to administer various doses of MDMA (5 to 20 mg/kg) given one to four times per day over a short period of successive days (Harper, Kay, & Hunt, 2013). This strategy is thought to allow translation to human recreational use. For example, with 5 mg/kg administered three times at two hour intervals the peak plasma concentration in rodents is around 700 ng/mL, which closely resembles plasma concentrations during a high-dose binge of MDMA in recreational users (Rodsiri, Spicer, Green, Marsden, & Fone, 2011).

Locomotor activity.

Behavioural sensitisation or tolerance in locomotor activity may develop following MDMA exposure, which may depend on whether previous exposure is repeated intermittent dosing or a single high dose (Schenk & Bradbury, 2015).

Several studies have demonstrated that multiple injections of MDMA of various doses for 3-5 days results in augmentation of locomotor activity to a further MDMA injection given

up to 4 weeks later when compared with controls. Sensitisation to the psychomotor stimulating effects of this drug is consistent with other abused psychostimulants (Ball, Wellman, Fortenberry, & Rebec, 2009; Bradbury, Gittings, & Schenk, 2012; Kalivas et al., 1998; McCreary et al., 1999; Spanos & Yamamoto, 1989). Repeated administration of MDMA enhances the capacity of the drug to elevate extracellular dopamine in the NAc, indicating that the sensitising effects of chronic exposure may be due to augmented DA neurotransmission (Kalivas et al., 1998). Consistent with this hypothesis, the sensitised locomotor response has been shown to be preferentially expressed in the centre of the box, resembling the locomotor activity that is produced by amphetamines (Bradbury et al., 2012; McCreary et al., 1999). This may also reflect tolerance to the anxiogenic properties of MDMA.

Other studies have produced conflicting results. A study by Kindlundh-Hogberg et al. (2007) used three injections of 5 mg/kg (3 h apart) every week for four weeks in an attempt to model weekend-binge ecstasy use in humans. They found that although MDMA cause reduced SERT density in the NAc, there were no changes in the horizontal activity between weeks suggesting neither tolerance nor sensitisation (Kindlundh-Hogberg et al., 2007). Alternatively, Brennan and Schenk (2006) found that pre-treatment with a single high dose binge of MDMA (4 doses of 10 mg/kg 2h apart) resulted in behavioural tolerance to MDMA with reduced locomotor activity and a downward shift of the dose-response curve two weeks later. This effect was most likely due to impaired 5-HT neurotransmission as previously described. Tolerance was not apparent after twelve weeks, suggesting that the neuroadaptations to a single high dose binge of MDMA were reversible (Brennan & Schenk, 2006).

Anxiety.

It has been consistently demonstrated that repeated administration of MDMA can cause changes in anxiety-like behaviour in rats and mice for up to three months after exposure (Faria

et al., 2006; McGregor et al., 2003; Mechan et al., 2002; Morley, Gallate, Hunt, Mallet, & McGregor, 2001; Piper & Meyer, 2004). However, few studies have looked at whether there is tolerance or sensitisation following repeated exposure of MDMA on measures of anxiety.

While the psychomotor activating effects of MDMA may become sensitised following repeated administration, there may be tolerance to the anxiogenic effects. Repeated administration has been shown to produce a shorter emergence latency from a dark box into an open field (Jones et al., 2010). Initial neophobia to open spaces observed with acute MDMA administration therefore seem to be attenuated after repeated exposure. The attenuated anxiogenic effect of MDMA following subchronic dosing is likely due to depletions of 5-HT, since repeated MDMA administration has been shown to prevent the anxiogenic effect of a 5-HT_{2A} receptor agonist in the elevated plus maze (Bull, Hutson, & Fone, 2004). Together it seems that there is acute tolerance to the anxiogenic effects of MDMA after repeated exposure. The current thesis will use the light/dark box to assess whether these findings can be replicated for MDMA and whether there is a similar behavioural tolerance for methylone.

1.5.2 Effect of repeated exposure to methylone.

Repeated administration of methylone in rats (3 or 10 mg/kg every 2 h for 3 doses) did not cause any reduction in cortical or striatal monoamine neurotransmitters two weeks after administration (Baumann et al., 2012). In contrast, at higher binging levels there has been shown to be a significant widespread depletion of 5-HT and 5-HT transporter levels in rats for up to two weeks post-administration (den Hollander et al., 2013; Lopez-Arnau, Martinez-Clemente, Pubill, Escubedo, & Camarasa, 2014). This is consistent with the effects of repeated administration of MDMA.

Repeated doses of methylone in mice followed by eight weeks of abstinence does not appear to affect locomotor activity, working or spatial memory in the T-maze, or anxiety as

measured by the elevated plus maze (den Hollander et al., 2013). Methylone treated rats did not demonstrate spatial learning deficits one week after binge exposure in the Morris Water Maze, although they displayed poorer performance in a single probe trial 24 h after the acquisition phase suggesting impaired reference memory (Lopez-Arnau et al., 2014). This is consistent with the finding that binge doses of methylone cause depletion of 5-HT in rats but not mice (den Hollander et al., 2013), and suggests a role for 5-HT in this memory task.

No human or animal behavioural studies currently exist that have examined whether there is development of tolerance or sensitisation to the effects of methylone following repeated exposure. This will be examined in the current thesis.

1.6 Adverse Effects and Hyperthermia

1.6.1 Adverse drug effects of MDMA.

Acute toxicity from MDMA ingestion relates to neuroendocrine, thermoregulatory, and cardiovascular systems. In a study of emergency department admissions due to the use of MDMA, the most severe complications were hyperthermia, hyponatraemia, rhabdomyolysis, cerebral oedema and coma, although the estimated ecstasy-related morbidity rate in recent users was very low (Halpern et al., 2011). It is important to note, however, that MDMA exhibits non-linear pharmacokinetics in human users, with a three-fold increase in dose of MDMA causing a six-fold increase in peak plasma concentration, suggesting that small increases in dose may lead to a disproportionate rise in plasma concentration, and thereby increasing the risk of acute toxicity (de la Torre, Farre, Ortuno, et al., 2000; de la Torre, Farre, Roset, et al., 2000; Mas et al., 1999).

In rats, MDMA can produce signs of neural excitability with piloerection, hypersalivation, and urination, which are seen in oral doses above 25 mg/kg. At doses up to 300 mg/kg there are tremors, convulsions, and death (Shulgin, 1986; Spanos & Yamamoto, 1989). The LD_{50} is considerably lower for intraperitoneal administration, with the LD_{50} in Sprague-Dawley rats found to be 49 mg/kg (i.p.) (Hardman, Haavik, & Seevers, 1973).

Hyperthermia.

One of the most well recognised adverse event that can follow MDMA intoxication in recreational users is hyperthermia, with temperatures of up to 43^{o}C being reported (Henry, Jeffreys, & Dawling, 1992). Hyperthermia is particularly concerning since this can precede DIC, rhabdomyolysis, and multi organ failure, with the majority of adverse reactions with core temperatures over 42^{o}C being fatal (Cole & Sumnall, 2003b).

In the rat, MDMA administration has generally been shown to cause a significant increase in core temperature of up to 2^{o}C in a dose-dependent manner (Docherty & Green, 2010; Green et al., 2003). This response has been shown in several studies to be dependent on ambient temperature. At normal or high ambient temperatures (> 20^{o}C) MDMA causes a hyperthermic response, while at low ambient temperatures (< 17^{o}C) MDMA has been shown to produce hypothermia (Green, O'Shea, Saadat, Elliott, & Colado, 2005). The hyperthermic effect was also found to be potentiated by social interaction (Kiyatkin, Kim, Wakabayashi, Baumann, & Shaham, 2014).

Interestingly, in humans trials MDMA does not reliably cause an increase in core body temperature (Grob, Poland, Chang, & Ernst, 1996; Mas et al., 1999; Vollenweider et al., 1998), although significant mild increases in body temperature (< 1^{o}C) have been recorded in some studies at higher doses of MDMA (Freedman, Johanson, & Tancer, 2005; Liechti et al., 2001; Parrott, 2012). It is likely that the hyperthermic response in human users is amplified in the

environments where MDMA is likely to be consumed, such as raves and night clubs where there is overcrowding, high ambient temperatures, and where they tend to consume little water together with considerable alcohol (Cole & Sumnall, 2003a; Green et al., 2003).

The primary mechanism for MDMA induced hyperthermia in the rat appears to be impaired heat loss mechanisms due to sustained peripheral vasoconstriction, which prevents proper heat dissipation to the external environment (Kiyatkin et al., 2014). This would explain why changes in body temperature seen in rats after MDMA administration is so dependent on ambient temperature. However, the effect of MDMA on body temperature is complex due to the widespread actions of this drug on multiple neurotransmitter systems and their effects on both central thermoregulation and peripheral changes in blood flow (Docherty & Green, 2010).

While cases of severe acute hyperthermia with MDMA are relatively unusual, the consequences can be fatal (Parrott, 2012). Since hyperthermia is related to significant toxicity, it is important to ascertain whether methylone also produces a similar temperature response which could have implications on its safety profile in human users.

1.6.2 Adverse drug effects of methylone.

Low recreational doses of synthetic cathinones enhance mood and increase energy, while high doses can cause serious medical complications. In humans, acute intoxication with methylone has been reported as causing seizure-like activity and hyperthermia leading to death, where peripheral blood concentrations were in excess of 0.5 mg/L (Pearson et al., 2012). In rats, methylone at high intraperitoneal doses (56 mg/kg) has been shown to cause tremors and convulsions (Marusich et al., 2012) and lethality within 1 hour at 60 mg/kg (den Hollander et al., 2013).

Several studies have demonstrated a significant effect of methylone on body temperature in mice and rats (Baumann et al., 2012; den Hollander et al., 2013; Kiyatkin, Kim, Wakabayashi, Baumann, & Shaham, 2015). Multiple subcutaneous injections of methylone in rats (3 doses of 3 to 10 mg/kg 2 h apart) has been found to increase core temperature, but to a much lesser degree than that of MDMA (Baumann et al., 2012). Den Hollander et al., (2013) found that intraperitoneal doses of methylone at 30 mg/kg consistently produced a 2^{o}C increase in body temperature in both mice and rats, as measured with a rectal probe (den Hollander et al., 2013). A recent study by Kiyatkin et al. (2015) looking at temperature changes in Long-Evans rats administered with various doses of methylone (1 and 9mg/kg) found that methylone dose-dependently increased brain (NAc) temperatures by less than 2^{o}C, which may be partly due to the peripheral vasoconstriction produced by the drug. This effect lasted for up to 4.5 hours post administration. Unlike MDMA, there was no evidence of potentiation of this hyperthermic response by increased ambient temperature or during social interaction (Kiyatkin et al., 2015).

1.7 Behavioural Tests Used in the Current Study

In order to characterise the acute effects of MDMA and methylone, a wide battery of behavioural tests was used focussing on three domains: locomotor activity, anxiety, and memory. The tests used were selected based upon previous research on MDMA, as previously discussed.

1.7.1 Open field test.

The open field test is was originally developed by Hall (1934) to measure emotionality in rats. Since then it has become useful in observing a range of behaviours that relate to

psychomotor activity, anxiety, and exploration. In this test the animal is placed in an open Perspex box and allowed to move freely about the chamber for a designated amount of time. A variety of behaviours can then be observed and recorded. In the current study, recorded behaviours were horizontal locomotor activity (ambulation), the amount of time spent in the centre vs. periphery of the box, rearing behaviour, and number of faecal boluses.

Abused psychostimulants such as cocaine and methamphetamine produce dose-dependent increases in locomotor activity which reflects increased dopaminergic action in the NAc (Lopez-Arnau et al., 2012). Measurement of locomotor activity after administration of a drug is therefore an indirect measure of the activation of the reward pathways (Bubar et al., 2004). Psychostimulants that produce greater locomotor hyperactivation may therefore have higher abuse potential. Conversely, any drug that fails to enhance locomotor activity may have a reduced chance of being an abused substance (Gatch et al., 2013).

Avoidance of the central squares and suppression of rearing behaviour are useful indicators of anxiety. Avoidance of central squares and reduced rearing has previously been attributed to anxiogenesis (Jones et al., 2010; McCreary et al., 1999; Palenicek, Hlinak, Bubenikova-Valesova, Votava, & Horacek, 2007).

1.7.2 Light/dark box.

Tests of unconditioned anxiety in animal models is based on the conflict between the desire to explore novel environments and the tendency to avoid potentially dangerous environments, known as approach-avoidance behaviour (Kulesskaya & Voikar, 2014). The Light/Dark Box (LDB) test, developed by Crawley and Goodwin (Crawley & Goodwin, 1980), is a test for anxiety based on this approach-avoidance conflict which has been popular for

screening potential anxiolytic compounds (Kulesskaya & Voikar, 2014). During the test animals are placed in a dark box which has a door that leads into a brightly lit chamber. Animals are allowed to freely explore the protected (dark) and unprotected (bright) novel areas. This creates an approach-avoidance conflict between the animal's innate aversion to brightly illuminated areas, and their exploratory drive towards novel environments. Several parameters are often measured to provide an anxiolytic profile of the drug, including the number of transitions between compartments and time spent in each compartment (Hascoet & Bourin, 1998).

The light/dark box has become a commonly used test for evaluating the anxiolytic properties of drugs in mice and rats, where differences in exploration can reasonably be attributed to the effect of the drug. Thus, an increase in exploration time in the brightly lit chamber or an increase in transitions between compartments is indicative of anxiolytic activity (Bourin & Hascoet, 2003). A third measure of anxiety in this test was the emergence latency time, or the time that it took for the rat to first move from the dark to the light area. Increased emergency latency is regarded as representing avoidance of a novel area, and therefore increased anxiety-like behaviour (Jones et al., 2010; Morley et al., 2005).

1.7.3 Novel object recognition task.

The novel object recognition (NOR) task (Ennaceur & Delacour, 1988b) measures non-spatial memory in the rat, and is based on their spontaneous tendency to explore novel objects (Camarasa et al., 2008). In a standard NOR there is an acquisition trial which consists of exploration of two identical sample objects. After a delay, one of the objects is replaced with a novel object and exploration time of the novel object is measured, along with total exploration in both the acquisition and recognition phase. An increase in the relative time exploring the

novel object is seen as a measure of working memory. It has the advantage that it is quick and simple to perform, and has therefore been useful in assessing the short and long term effects of drugs of abuse on memory (Schenk et al., 2011). Since this task requires no previous reinforced training it is a more pure measure of working memory, with relatively little reference memory (Harper et al., 2013).

1.8 Rationale and Hypotheses

While there are a small number of studies that have examined the pharmacological aspects of methylone, few studies to date have examined the behavioural correlates of this potential substance of abuse. To our knowledge, no study exists that comprehensively compares the behaviour of methylone to MDMA, whose subjective effects methylone is said to closely resemble. In addition, no studies exist that look at the behavioural effects of methylone after repeated administration to determine if there is tolerance or sensitisation of the acute behavioural effects.

Due to the ongoing high rates of abuse of MDMA and methylone, it is important to determine the acute and long-term effects of these drugs on health and neuropsychological functioning. Scheduling and controlling methylone may be warranted given its abuse potential, known psychostimulant effects, and implication in acute toxicity and death in recreational users. However, classification of this drug without pre-clinical behavioural data is problematic. Dose-response data are desirable for almost all new substances that are used either medically or recreationally by humans in order to determine "safe" and "hazardous" dosage levels. The first part of this research will therefore investigate the behavioural effects produced by different dosages of methylone in rats, and in particular, how similar they are to an equivalent dosage of MDMA on tests of locomotor activity, memory, and anxiety as outlined above. Since the

psychopharmacological response of methylone on extracellular monoamine levels is less than that of MDMA, and monoamine levels are considered to be responsible for the behavioural effects of amphetamine derivatives, it is hypothesised that methylone will have a dose-response characteristic that is approximately half that of MDMA. In addition, it is hypothesised that both MDMA and methylone will show significantly greater behavioural effects in female rats, consistent with previous research demonstrating greater locomotor effects in females for MDMA (Palenicek et al., 2005; Walker et al., 2007). Specifically it is hypothesised that both MDMA and methylone will cause enhanced locomotor activity confined to the periphery and reduced rearing activity. It is further hypothesised that MDMA and methylone will produce anxiogenic behaviours in the LDB and working memory impairments in the NOR task.

Next this research will assess whether methylone produces behavioural tolerance or sensitisation similar to MDMA by injecting rats with a high binge dose of either MDMA or methylone and assessing their subsequent behaviour to a further challenge of the respective drug one week later. Behavioural sensitisation reflects many aspects of drug addiction, including the propensity of addicts to relapse (Ball et al., 2009). If the positive mood-altering effects of a drug are reduced following repeated exposure it may result in greater consumption of the drug per session, which can result in increased adverse effects of the drug, such as cognitive and psychiatric problems, and also increases the likelihood of overdosing with potentially harmful medical sequelae. Due to the similar psychopharmacological action and chemical structure between MDMA and methylone, it is hypothesised that methylone will produce a similar rapid behavioural sensitisation to the psychomotor activating effects and tolerance to the anxiogenic effects, as is observed from repeated exposure to MDMA. In addition, temperature measurements will be taken after acute intoxication, given that hyperthermia may potentiate the neurotoxic effects of drugs of abuse (Docherty & Green, 2010).

Finally, there are a limited number of studies that have looked at sex differences in the effects of MDMA. These studies have consistently shown that female rats are more sensitive to the locomotor stimulating effects of MDMA, consistent with the enhanced response of female rats to other psychostimulants, including cocaine and amphetamine (Palenicek et al., 2005; Walker et al., 2007). This has important implications in terms of subjective and adverse effects of these drugs in female users, and may indicate an increased risk of physical or psychological harm. It is currently unknown whether similar sex differences occur following methylone intake. The current study will therefore use both male and female rats in order to confirm these findings for MDMA and to determine if similar sex differences exist with methylone.

Method

2.1 Animals

A total of 108 (54 male and 54 female) naïve adult PVG/C hooded rats (University of Canterbury, New Zealand) aged between 199 and 278 days old were used in the experiments. The use of 12 rats for each of the conditions was required in order to achieve more than 80% power. Both male and female rats were used since significant sex differences in the effects of acute MDMA administration in rats has been previously reported (Koenig et al., 2005; Palenicek et al., 2005). The weight of the animals ranged from 195 and 400g at the start of experimentation. The rats were housed in groups of 3-4 in large cages in a temperature controlled environment (21^{o}C+/-1^{o}C), with food and water ad lib. A 12h light/dark cycle was in operation and all testing took place during the light cycle. Experimentation was approved by the University of Canterbury Animal Ethics Committee.

2.2 Drugs

Methylone ((±) 2-methylamino-1-[3,4-methylenedioxyphenyl]propan-1-one) and MDMA ((±) 3, 4-methylenedioxymethamphetamine hydrochloride) were obtained from BDG Synthesis (Wellington, NZ) and were shown to have greater than 99% purity by HPLC. Methylone and MDMA solutions were prepared at various concentrations (2.5mg/mL, 5mg/mL, 8mg/mL, and 12mg/mL) by dissolving 25mg, 50mg, 80mg, or 120mg of powder in 10mL of normal saline (0.9% NaCl w/v), respectively. Normal saline alone was used as the control solution. These solutions were then transferred to air-tight injection vials, and were labelled and stored at room temperature in a dark safe.

2.3 Procedure

2.3.1 Procedure for the acute behavioural effects of MDMA and methylone.

The 108 rats were randomly assigned to one of 9 experimental conditions; MDMA 2.5mg/kg, MDMA 5mg/kg, MDMA 8mg/kg, MDMA 12mg/kg, Methylone 2.5mg/kg, Methylone 5mg/kg, Methylone 8mg/kg, Methylone 12mg/kg, or vehicle (saline 0.9%), with 12 rats per condition (6M and 6F).

2.3.1.1 Drug Administration.

On experimental days, groups of 9 rats (either 1 M or 1 F for each condition) were weighed and then returned to their home cage. Drug doses were calculated for each rat (at 1mL/kg), and appropriate volumes of each of the drug solutions were drawn into 1mL i.p. injection syringes and were labelled. Twenty minutes prior to testing, individual animals were removed from their home cage, injected intra-peritoneally in the right lower quadrant of the abdomen with the appropriate syringe, and were then returned to their home cage. All experiments were done in standardised laboratory conditions, under a normal light/dark cycle, at an ambient temperature of 21±1^{o}C and an average light level of approximately 360 lux.

2.3.1.2 Behavioural Testing.

On the first day of testing, rats received the appropriate dose of drug and were subsequently tested in both the open field test and light/dark box test (*2.3.1.1.* and *2.3.1.2*). One week later rats were injected again with the same dose of drug and were tested in the object recognition task (*2.3.1.3*). Half of the rats were counterbalanced and were tested in the object recognition test on the first day of testing and open field test and light/dark box test one week later. The observer scoring the behaviour was blinded to condition.

Open Field Test.

Twenty minutes post-injection each animal was placed in a novel open field (610 x 610 x 250 mm) with clear Perspex sides and a black plastic floor which was divided into 16 square blocks (each 152mm x 152mm) which was outlined on the field floor in white paint. Each block corresponded to a spatial location designated by an (x,y) parameter, which ranged from (1,1) for the bottom left corner to (4,4) for the top right corner (*Figure 2.1*). The rat was placed in the centre of the open field facing away from the observer, and a timer was started. Open field activity was recorded by an observer every 3 seconds for 5 minutes on a scoring sheet by noting down the spatial location of the rat (the square in which the greatest proportion of the rat's body was at the time of the recording), and whether the rat was displaying any rearing activity at that time. If the rat did not change location a dash (-) was noted.

(1,4)	**(2,4)**	**(3,4)**	**(4,4)**
(1,3)	**(2,3)**	**(3,3)**	**(4,3)**
(1,2)	**(2,2)**	**(3,2)**	**(4,2)**
(1,1)	**(2,1)**	**(3,1)**	**(4,1)**

FRONT

Figure 2.1. Aerial view of base of the open field with spatial location parameters. The lighter shaded region represents the centre of the open field.

Following testing the rat was returned to its cage and the testing arena was cleaned by spraying the floor and sides with Powerquat Blue (2%) and wiping down with a paper towel. This was to remove any olfactory cues left in the box between trials.

Path length was calculated by adding up the total number of blocks entered during the 5 minute trial and multiplying this by 15.2cm (length of one box) to give a total displacement (in cm). The total number of blocks entered was determined by calculating the x and y change between each 3 second interval, and summing these changes for all intervals.

Time spent in the centre was calculated for each rat by totalling the number of times that the rat was noted to be in the centre of the apparatus (lighter shaded region - *figure 2.1*) during the 5 minute trial, and multiplying this total by 3.

Rearing activity was calculated as the total number of times that the rat stood on its hind legs in an upright posture during the 5 minute trial.

Light/Dark Box Test.

The apparatus consisted of a clear varnished wooden box comprising two 300mm long x 200mm wide x 300 mm high compartments. The compartments were separated by a wooden partition with a 100mm x 100mm doorway that could be closed by means of a guillotine slide. One compartment was covered by a hinged wooden lid (dark side), and the other was covered by a hinged clear Perspex lid (light side). A camera was situated 50cm above the top of the box and was projected onto a 13” TV screen which was situated next to, and facing away from, the light/dark box.

Immediately following the open field test (approximately 25 minutes post-injection) the rat was placed into the dark side of the light-dark box and, 10 seconds later, the guillotine slide was removed. The observer sat 2 m from the apparatus facing the TV screen so that they were obstructed from view of the rat. By means of a hand-held counter and timer, the observer recorded the emergence latency from the dark side (time until all 4 paws of the rat are placed into the light side), the number of transitions between the two sides, and the total time spent in the light side. At the end of the 5-min trial, the rat was returned to its home cage, and both sides of the box were cleaned by spraying the floor and sides with Powerquat Blue (2%) and wiping down with a paper towel.

Object Recognition Test.

Testing took place in a square wooden arena (600 x 600 x 250 mm) which had black painted walls and floor. A miniature video camera was situated above the arena which was projected onto a 13" TV screen. The TV was viewed by the observer so that they were obstructed from view of the rat. Behaviour was observed and analysed in real time by the observer. The duration of exploration of each of the objects was measured by stopwatch, where exploration was defined as directing the nose at a distance of less than 2 cm to the object and/or touching it with the nose (Ennaceur & Delacour, 1988a).

One week after the open field test and light/dark box testing, rats were habituated to the testing arena for 2 minutes, after which they were removed, injected with the appropriate dosage of drug, as outlined above, and returned to their home cage. Twenty minutes post-injection, rats were exposed to two identical objects in the arena for a 5 minute acquisition period, and then returned to their home cage. After a 15 minute inter-trial delay, rats were placed back in the

arena for a 3 minute trial, with the original objects being replaced with one identical and one novel object. The objects used were a green plastic cup (which was half-filled with sand to prevent the rats from knocking it over) and a large black plastic lid with a hollow centre. The two sets of objects were used during each test session and the objects used as the "original' and "novel" objects were counterbalanced within and across groups to avoid potential object-preference bias.

2.3.2 Procedure for repeated drug exposure.

One week following behavioural testing (*section 2.3*) the drug naïve rats and rats from the lowest dose (2.5 mg/kg) drug groups were used to assess the level of biological tolerance of methylone and MDMA. Animals were used from the lowest dose group of prior drug administration to ensure that any carry-over effect from previous exposure was minimal.

2.3.2.1 Drug Administration.

Both MDMA and Methylone were dissolved in normal saline (0.9% NaCl w/v) at a concentration of 5.0 mg/mL and were injected intraperitoneally (i.p) at a volume of 1 mL/kg. Rats from the MDMA group were administered with 5.0 mg/kg of MDMA every hour for 3 h on each of 2 consecutive days to give a total cumulative dose of 30 mg/kg. Rats from the methylone group received the same dosing regimen using methylone 5.0 mg/kg. Control rats received equivalent injections of 0.9% saline.

2.3.2.2 Body temperature.

Body temperature of the rats was recorded every hour immediately following each administration of the drug, and 1 hour after the last injection. Body temperature was measured

using a Braun ThermoScan ExacTemp Thermometer (IRT 4020). During recording the probe of the thermometer was placed into the external auditory meatus and held until reading of the tympanic membrane temperature was completed (approx 3 s). Temperature was recorded twice in each ear and the readings were averaged. This tympanic temperature reading technique has been shown to provide an accurate and rapid measure of core temperature in rats that is highly correlated with rectal temperature (Morley et al., 2001).

2.3.2.3 Behavioural Testing.

One week following drug administration, and 20 minutes prior to behavioural testing, rats received a 5 mg/kg challenge injection of MDMA, methylone, or equivalent volume of 0.9% saline, and were returned to their home cage. Twenty minutes post-injection, rats were tested in the open field and light/dark box as outlined previously, with counterbalancing between the two tests. Following testing all rats were returned to their home cage.

2.4 Statistical Analysis

Data was analysed using Statistica 10 software package for windows. T-tests for independent means were carried out between drugs for each behavioural test to determine if there were overall significant differences in the effects of each drug. Factorial ANOVAs were calculated for each drug for each of the behavioural tests. When significant main or interaction effects were found, *post-hoc* comparisons were made using one-tailed Dunnett's tests to compare all doses with saline control, and Tukey's HSD to establish pair-wise comparisons between various doses. All analyses were completed with an alpha value of .05.

Results

3.1 Acute Behavioural Effects of MDMA and Methylone

3.1.1 Open field test.

Descriptive statistics for displacement, time in centre, and number of rearings were calculated by drug type for all rats. T-tests for independent means were calculated between the means of the MDMA and methylone groups for each measure (Table 3.1).

One of the male rats in the 12mg/kg group died following injection, and was therefore unable to be used to complete the open field test. The data used for analysis contained data from 12 rats per group (6M and 6F), with only 11 rats in the 12mg/kg MDMA group (5M and 6F).

There was a significant difference between the MDMA and methylone groups on the measure of time spent in centre, with MDMA rats spending significantly more time in the centre than methylone rats. There was also a significant difference in the number of rearings with MDMA rats having significantly less rearing behaviour than methylone rats. There was no significant difference in the mean displacement between MDMA and methylone while collapsing across dose.

Two-way factorial ANOVAs were used to calculate effects of each drug (MDMA and methylone) for each of the behavioural tests in the Open Field, with dose and sex as the independent factors. Assumptions of normality and homogeneity of variance were assumed to be satisfied given that the methodology was appropriate for the investigations, and there was independence between observations. In addition, the two-way ANOVA is known to be robust against violations in normality and homogeneity of variance when sample sizes are equal within groups, as there was in the current experiment. Therefore small violations of these assumptions are not likely to interfere with results. (reference?)

Table 3.1. *Means and Standard Deviations of Open Field Responses with T-tests Between the Means of all MDMA and all Methylone Groups Collapsed for Each Test.*

Open Field Tests	**Saline (N=12)**		**MDMA (N=47)**		**Methylone (N=48)**		***T*-tests**	
	Mean	Std. Dv.	Mean	Std. Dv.	Mean	Std. Dv.	*t*-tests	p
Displacement (cm)	902.3	296.2	2125	137.6	2460	143.7	-1.68	0.10
Time in Centre (s)	34.00	18.51	63.38	37.73	48.38	30.83	2.13	0.036*
Rearings (N)	30.00	9.46	5.19	6.53	14.29	12.43	-4.45	0.000**

* significant to $p < 0.05$

** significant to $p < 0.01$

Displacement.

Descriptive statistics for displacement can be found in *table 3.1*. Factorial two-way ANOVAs were calculated for each drug (MDMA and methylone), with displacement as the dependent variable and dose and sex as the independent factors. There was a significant main effect of dose for MDMA ($F(4, 49) = 5.04$, $p = .002$) and methylone ($F(4, 50) = 17.44$, $p < .001$), with increasing doses of each drug producing a greater displacement (*fig. 3.1*), consistent with the hypotheses. Post-hoc comparisons using Dunnett's test showed that all doses of MDMA and methylone produced significantly greater displacement than saline vehicle. Tukey's test showed no significant differences between doses for MDMA, but demonstrated that 12mg/kg methylone had significantly greater displacement than low doses (2.5 and 5mg/kg), indicating that higher doses of methylone continued to increase displacement above lower doses, while MDMA reached its maximal effect on displacement at 2.5mg/kg. A significant main effect was found for sex for both MDMA ($F(1, 49) = 4.80$, $p = .033$) and methylone ($F(1, 50) = 7.32$, $p = .009$), with females having a significantly higher level of displacement in both groups (*Figure 3.1*). This is consistent with previous research demonstrating a greater effect of MDMA on psychomotor stimulation in female rats, and suggests that methylone also has a significantly greater stimulant effect on females. There were no significant sex X dose interactions for either drug group. While the difference between drug groups overall did not reach significance (Table 3.1), a t-test for independent means between the high dose groups (8 and 12mg/kg) of each drug showed a significant difference ($t(45) = -2.39$, $p = .021$), with methylone causing higher levels of displacement than MDMA. This indicates that the maximal effect of methylone on psychomotor stimulation was greater than that of MDMA.

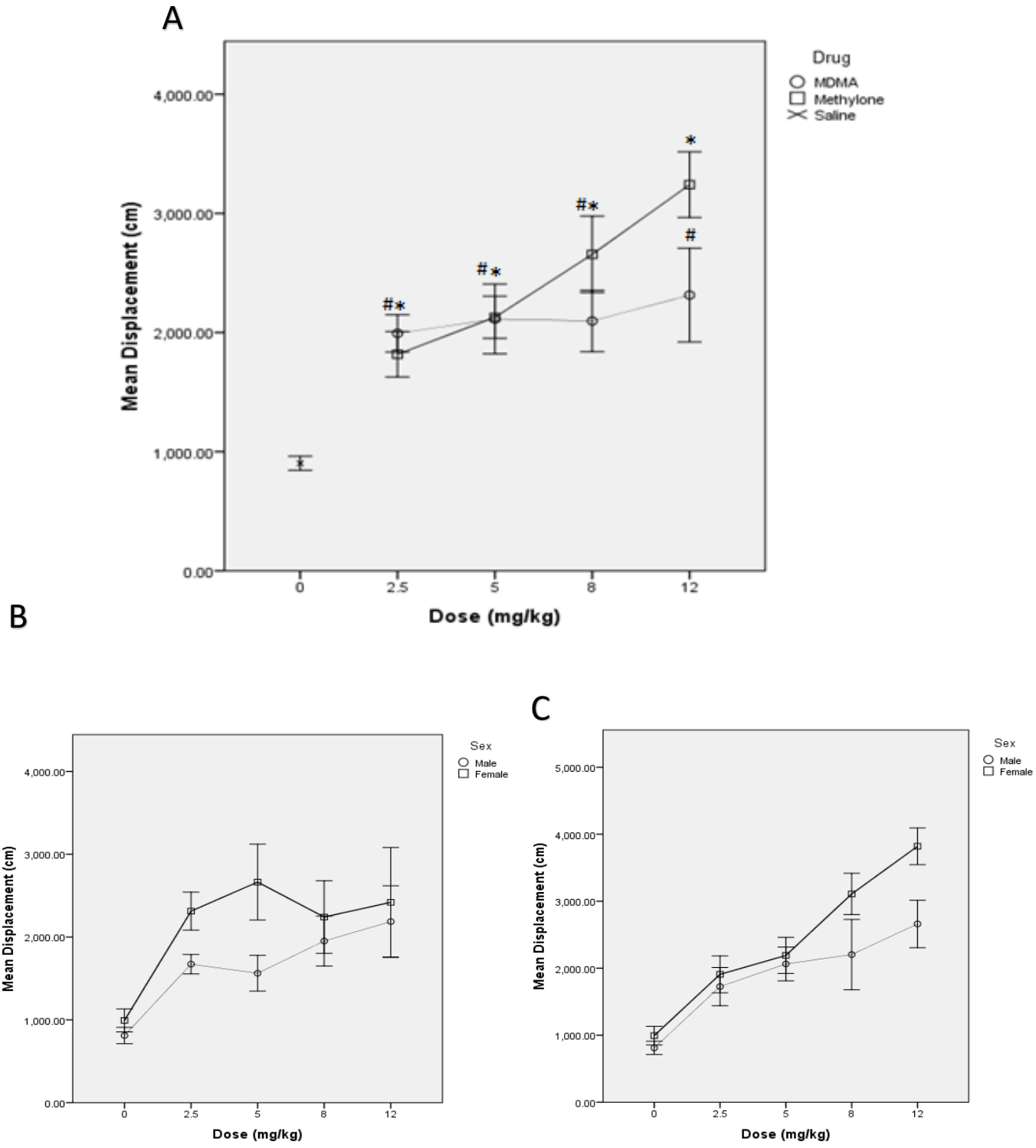


Figure 3.1. Mean displacement (cm) in the open field for MDMA and methylone treated rats by dose of drug administered (mg/kg). ***A.*** *Mean (± SEM) displacement for rats injected with either MDMA, methylone, or saline vehicle. * p<.05 methylone compared with saline; # p<.05 MDMA compared with saline* ***B****. Mean (± SEM) displacement for male and female rats administered MDMA.* ***C.*** *Mean (± SEM) displacement for male and female rats administered methylone.*

Time in centre.

The spatial characteristics during each trial were determined by measuring the amount of time the rat spent in the centre four squares of the open field. A summary of the descriptive statistics for time spent in the centre can be found in Table 3.1. Factorial two-way ANOVAs, with time spent in the centre as the dependent variable and sex and dose as independent factors, were calculated for each drug. A significant main effect of dose was found for MDMA ($F(4, 49) = 2.59$, $p = .048$), with increasing doses of MDMA causing an increase in the time spent in the centre (*Figure 3.2*), contrary to the original hypothesis. Post-hoc contrasts with Dunnett's test showed that only high doses (8 and 12mg/kg) were significantly different from saline control, indicating that MDMA-induced changes in spatial characteristics of locomotion were only produced at higher doses. There was no main effect for methylone ($F(4, 50) = 0.63$, $p = 0.646$) suggesting that there was no effect of this drug on the spatial characteristics of movement about the chamber. There were no significant main effects of sex for either drug, and there were no significant sex X dose interactions.

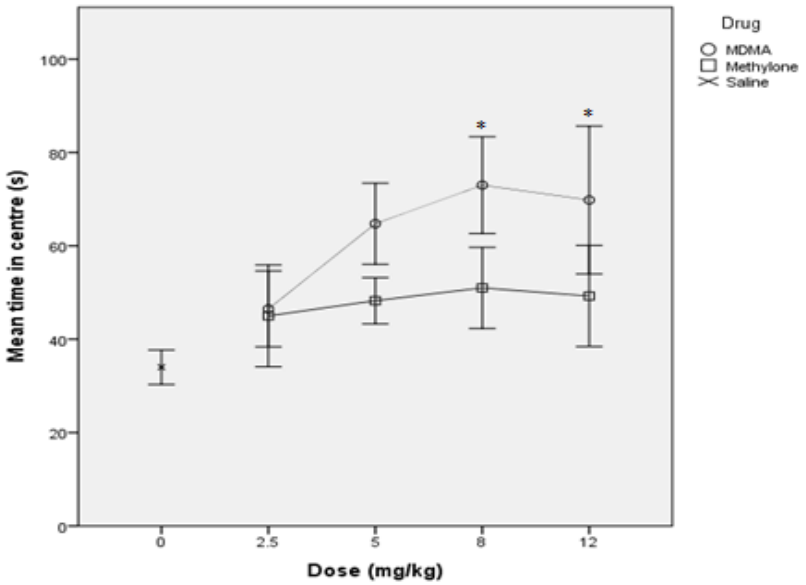


*Figure 3.2. Mean time spent in the centre of the open field (s) for MDMA and methylone treated rats by dose administered (mg/kg). * p < .05 compared to saline. Error bars denote ± SEM.*

Rearings.

Descriptive statistics for number of rearings can be found in Table 3.1. Factorial two-way ANOVAs, with rearings as the dependent variable and sex and dose as the independent factors, were calculated for each drug. There was a significant main effect of dose for both MDMA ($F(4, 49) = 44.36$, $p < .001$) and methylone ($F(4, 50) = 9.02$, $p < .001$), with each drug causing a significant decrease in rearing (*Figure 3.3*), consistent with the hypothesis. In addition, a t-test for independent means between MDMA and methylone showed that MDMA significantly reduced rearing activity more than methylone (Table 3.1). Post-hoc comparisons with Dunnett's test showed that all doses of MDMA significantly reduced rearing relative to saline control, but for methylone only doses of 5mg/kg or higher significantly reduced rearing. There was no significant main effect of sex or sex X dose interaction for either drug.

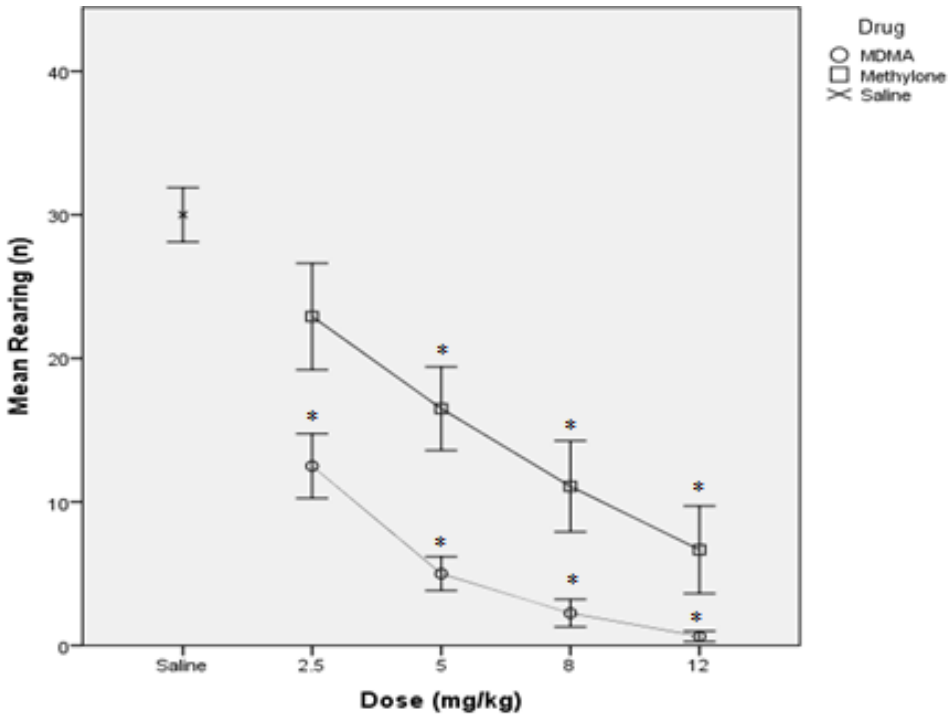


*Figure 3.3. Mean number of rearings in the open field for MDMA and methylone treated rats by dose administered (mg/kg). * $p < .05$ compared to saline. Error bars denote ± SEM.*

3.1.2 Light/Dark box.

Descriptive statistics for amount of time spent in the light (time in light), number of transitions between light and dark sides, and emergence latency (how long it took for the rat to first emerge from the dark side after the start of the trial) were calculated by drug type and can be found in Table 3.2. One of the male 12mg/kg rats died prior to testing, and was therefore excluded from analysis. Five rats failed to emerge from the dark box (1 from MDMA 8mg/kg, 2 from MDMA 12mg/kg, and 2 from Methylone 5mg/kg groups) and were also excluded from analysis. T-tests for independent means were conducted between the MDMA and methylone group means for each of the parameters, all of which were not significant (Table 3.2). Two-way factorial ANOVAs were used to analyse the light/dark box results for each drug, with time in the light side of the box, number of transitions between compartments, and emergency latency as the dependent variables, and with sex and dose as the independent factors.

Table 3.2. *Means and Standard Deviations of Light/Dark Box parameters with T-tests Between the Means of all MDMA and all Methylone Groups Collapsed for Each Test.*

Light/Dark Box Parameter	**Saline (N=12)**		**MDMA (N=44)**		**Methylone (N=46)**		***T*-tests**	
	Mean	Std. Dv.	Mean	Std. Dv.	Mean	Std. Dv.	*t*-tests	p
Time in light (s)	83.30	35.85	94.84	44.23	92.40	43.54	0.26	0.79
Total transitions (n)	8.75	2.18	16.52	9.73	14.72	8.28	0.95	0.35
Emergence Latency (s)	9.45	8.96	22.45	32.32	23.57	50.63	-0.12	0.90

* significant to $p < 0.05$

** significant to $p < 0.01$

Time in light.

While it appeared from the graph of mean time in the light vs. dose for each drug that there was a trend towards an increase in time spent in the light with increasing dose (*Figure 3.4*), there was no main effect of dose for either MDMA ($F(4, 46) = 1.27$, $p = 0.30$) or methylone ($F(4, 48) = 1.02$, $p = 0.41$). There was a significant main effect of sex for MDMA ($F(1, 46) = 8.31$, $p = .006$) with males spending more time in the light, on average, than females. There was no main effect of sex for methylone ($F(1, 48) = 3.13$, $p = 0.083$), and there were no significant sex X dose interactions for either drug. The current data suggest that although female rats spend more time in the light side of the box on average, there is no significant effect of either drug on total time spent in the light compartment.

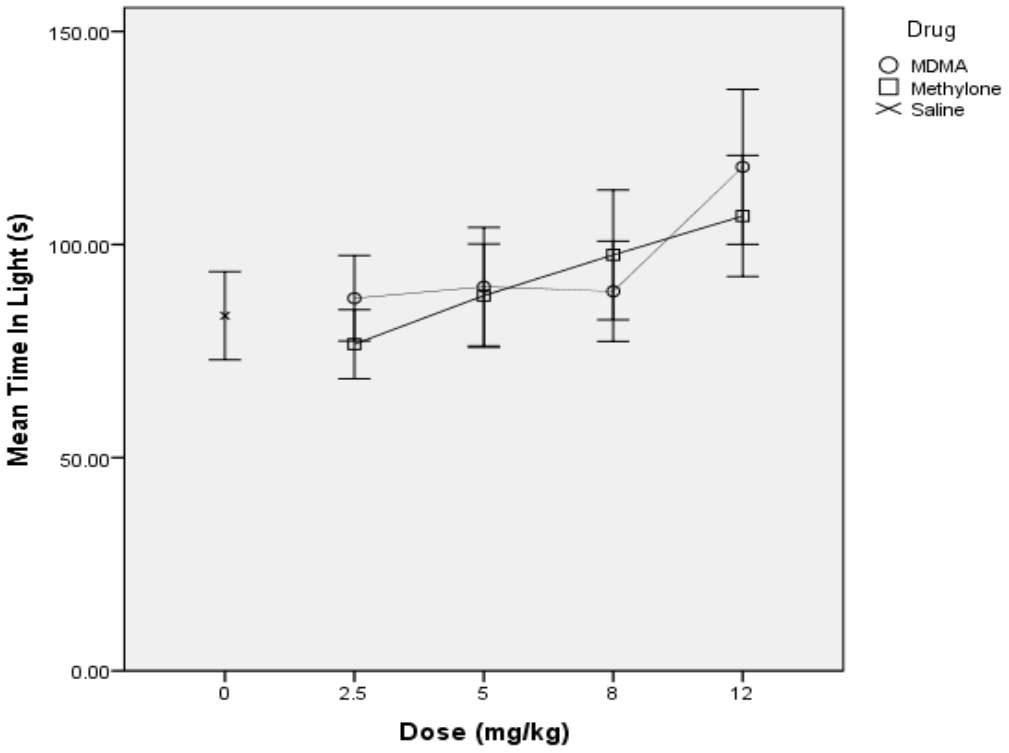


Figure 3.4. Mean time spent in the light compartment of the LDB (s) for MDMA and methylone treated rats by dose administered (mg/kg). Error bars denote ± SEM.

Transitions between light and dark compartments.

There was a significant main effect of dose for both MDMA ($F(4, 46) = 2.97$, $p = .029$) and methylone ($F(4, 48) = 4.35$, $p = .004$), with higher doses resulting in a greater number of transitions between the light and dark compartments (*Figure 3.5*). Post-hoc comparisons with Dunnett's test showed that there were only significant differences from saline control at 8 and 12mg/kg of MDMA, and 12mg/kg of methylone. This indicates that high doses of either drug cause a significant increase in transitions. There were no significant main effects of sex for either drug, and no significant sex X dose interaction effects.

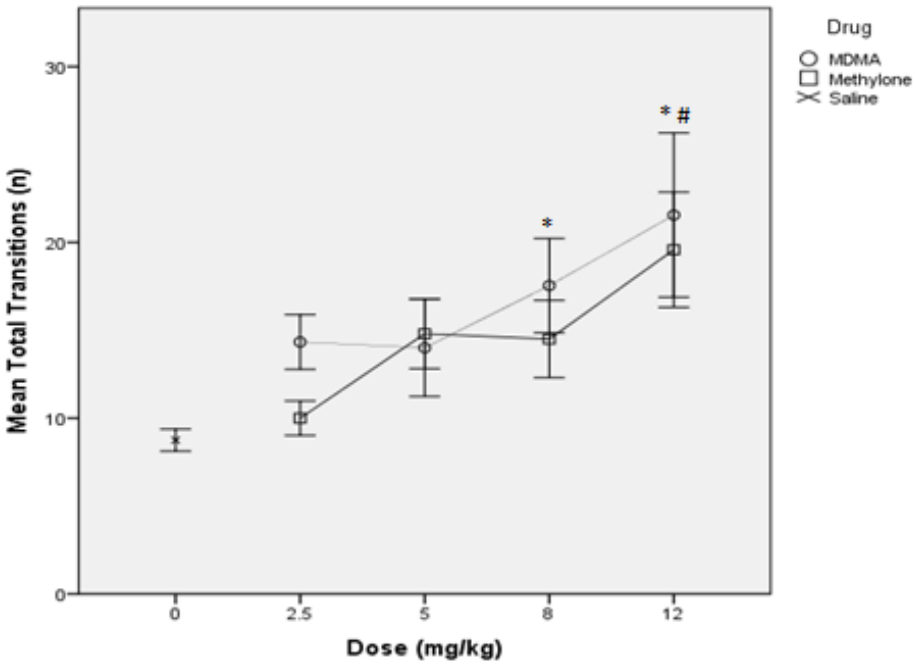


*Figure 3.5. Mean number of transitions between the light and dark compartments of the LDB for MDMA and methylone treated rats by dose administered (mg/kg). * p < .05 MDMA compared to saline. # p < .05 methylone compared to saline. Error bars denote ± SEM.*

Emergence Latency.

There was no main effect of dose on emergency latency in the ANOVA for either MDMA ($F(4, 51) = 1.27$, $p = 0.293$) or methylone ($F(4, 53) = 1.14$, $p = 0.349$), although the

graph of the mean emergence latency vs. dose indicated a trend towards increased latency with increasing doses of MDMA and methylone (*Figure 3.6*). Failure to reject the null hypothesis was likely due to the large standard errors for this parameter at higher doses. There was also no significant main effect of sex for either drug, and no significant sex X dose interaction effects. The current data therefore fails to demonstrate a significant difference in the emergence latency between drug-treated animals and saline controls.

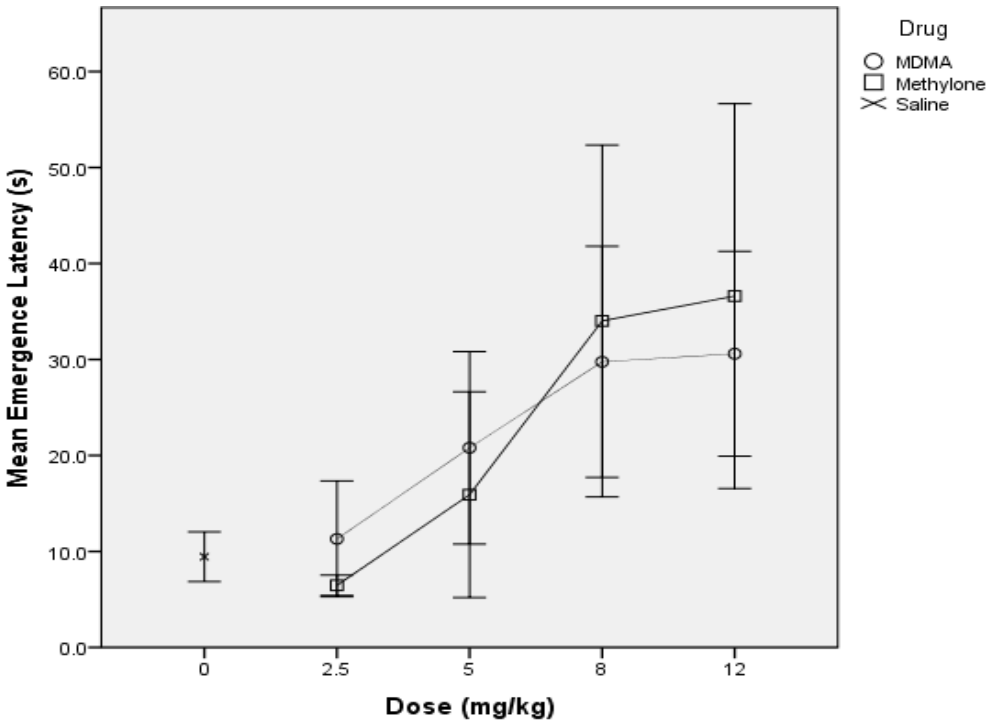


Figure 3.6. Mean emergence latency from the dark side of the LDB (s) for MDMA and methylone treated rats by dose administered (mg/kg). Error bars denote ± SEM.

3.1.3 Novel object recognition task.

During the object recognition task, the amount of time that the rat spent in exploring each of the novel (a) and identical (b) objects was recorded. Descriptive statistics for the mean exploration time for each drug are summarised in Table 3.3. Because one of the male 12mg/kg rats died prior to testing it was excluded from analysis. In addition, one of the female 8mg/kg

Table 3.3. *Means and Standard Deviations of Novel Object Recognition Task parameters with T-tests Between the Means of all MDMA and all Methylone Groups Collapsed for Each Test*

Object recognition parameter	**Saline (N=12)**		**MDMA (N=44)**		**Methylone (N=46)**		***T*-tests**	
	Mean	Std. Dv.	Mean	Std. Dv.	Mean	Std. Dv.	*t*-tests	p
Novel object exploration (s)	23.05	12.07	13.89	10.46	19.35	11.86	-2.263	.03*
Identical object exploration (s)	11.76	6.72	11.41	19.43	11.42	7.73	-0.003	0.997
Discrimination index	0.646	0.148	0.620	0.198	0.622	0.152	-0.046	0.963

* significant to $p < 0.05$

** significant to $p < 0.01$

MDMA rats failed to explore in this task and was therefore excluded. Novel and identical object times were used to calculate total exploration time (e = a + b), and a discrimination index (d_1 = a / (a + b)) where $d_1 > 0.5$ indicates higher discrimination between novel and identical objects, as previously described (Schenk et al., 2011). In addition, the side on which the novel object was placed (Left or Right) and the object chosen to be the novel item (Lid or Cup), were recorded, and t-tests were used to determine if there was place or object preference. Two-way ANOVAs were calculated for each drug, with total exploration time and discrimination as the dependent variables and sex and dose as the independent factors.

Object and place preference.

A t-test for independent means showed that there was a significant difference in mean novel exploration time between the lid (M = 14.60, SD = 10.88) and cup (M = 21.74, SD = 11.87), with the cup having a higher amount of novel exploration time ($t(210) = 3.273$, p = 0.001), indicating a preference for that object. There was no significant difference between means in total exploration time for side, indicating that there was no place preference.

Exploration time.

There was no main effect of dose on exploration time for either MDMA or methylone (*Figure 3.7*), indicating that these drugs did not impair exploratory activity, although there appeared to be a trend towards less total exploration time for MDMA treated rats at higher doses. There was a significant main effect of sex for both MDMA ($F(1, 48) = 4.93$, p = .032) and methylone ($F(1, 50) = 7.74$, p = .008), with females having a higher amount of total exploration time than males on average (*Figure 3.8*). There were no sex X dose interactions.

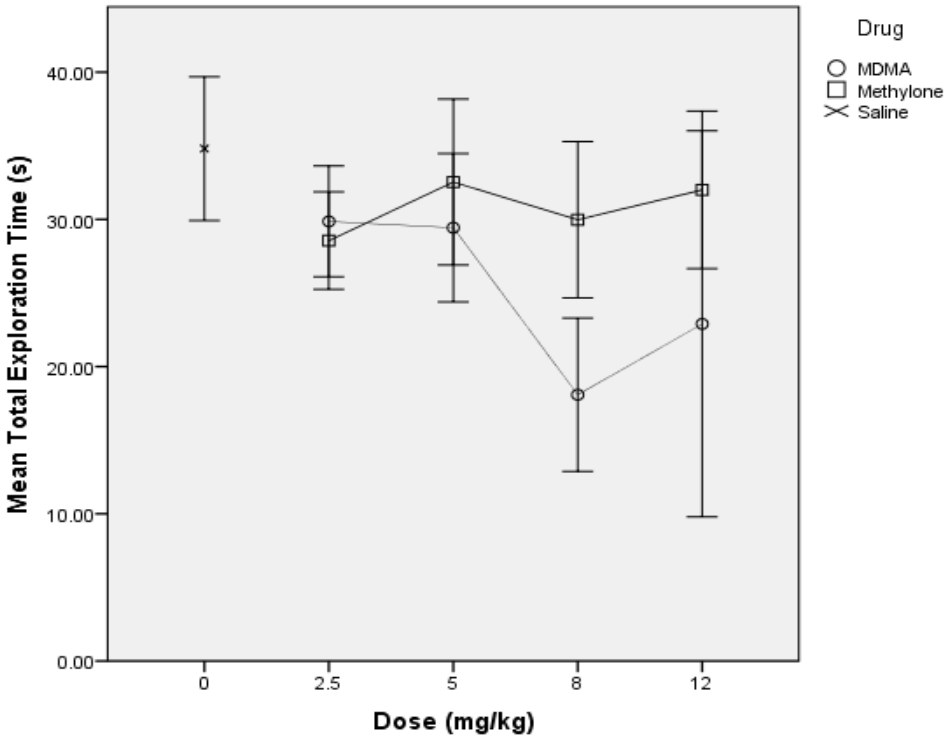


Figure 3.7. Mean total exploration time (s) of objects in the novel object recognition task (NOR) for MDMA and methylone treated rats by dose administered (mg/kg). Error bars denote ± SEM.

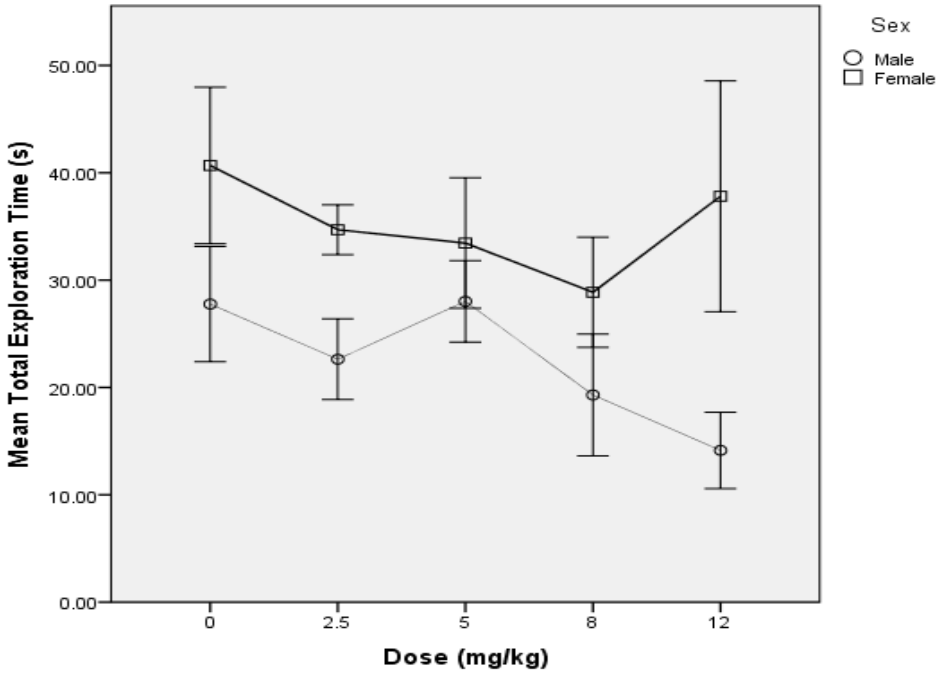


Figure 3.8. Mean total exploration time (s) of objects in the novel object recognition task (NOR) by dose administered for each sex averaged across drug type. Error bars denote ± SEM.

Object discrimination.

A single sample t-test showed that the discrimination between the novel and identical objects was significantly greater than 0.5 ($t(105) = 7.10$, $p < .001$), indicating that the rats were able to discriminate to a level greater than chance, on average. From the two-way ANOVAs there were no significant main effects of dose for either MDMA ($F(4, 48) = 0.45$, $p = 0.776$) or methylone ($F(4, 50) = 0.28$, $p = 0.888$) (*Figure 3.9*), indicating that there was no difference in object discrimination for rats exposed to either drug. There was also no main effect of sex for either drug. Since exploration time was also not affected by either drug, these results fail to demonstrate an impairment in working memory from acute methylone or MDMA exposure in this task.

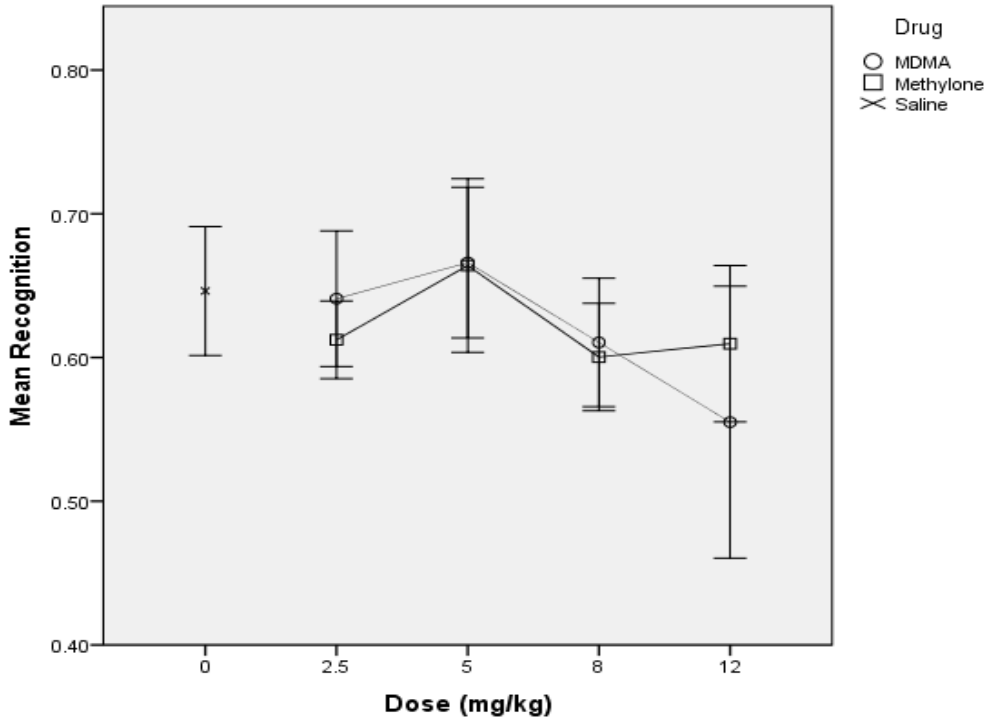


Figure 3.9. Mean discrimination index (d_1) in the object recognition task for MDMA and methylone treated rats by dose administered (mg/kg). Error bars denote ± SEM.

3.2 Temperature Change with Repeated Drug Administration

Temperature was recorded for 9 rats out of each drug group (4M and 5F) immediately at the time of the first injection (0 hours) and then at the time of each additional injection, and finally one hour after the last (third) injection, making a total of 4 measurements per rat. Means and standard deviations can be seen in Table 3.4.

Table 3.4. *Means and Standard Deviations of Temperatures of Rats During and After Repeated Administration of Saline, MDMA, or Methylone, With Zero Hours Being the Time of The First Injection.*

Hours	Saline		MDMA		Methylone	
	Mean	Std. Dv.	Mean	Std. Dv.	Mean	Std. Dv.
0	37.02	0.33	37.37	0.28	37.22	0.26
1	36.84	0.48	36.83	0.33	36.98	0.51
2	36.68	0.82	38.49	0.92	36.79	0.48
3	36.62	0.51	39.63	1.10	36.79	0.40

A repeated measures ANOVA, with temperature recordings as the repeated measure, time as the within group factor, and treatment (saline, MDMA, or methylone) as the between groups factor, showed a significant between-subject effect of treatment ($F(2, 23) = 8.22$, $p = .002$), a significant within-subject effect of time ($F(3, 69) = 5.97$, $p = 0.001$), and a significant Time X Treatment interaction ($F(6, 69) = 6.96$, $p < .001$). Linear contrast analysis showed that there was a significant increase in temperature for MDMA rats ($F(1, 23) = 123.24$, $p < 0.001$), but no significant change in temperature for methylone ($F(1, 23) = 3.82$, $p = 0.062$) or control rats ($F(1, 23) = 2.81$, $p = 0.106$) over time. The increase in temperature was not evident until 1 hour following the first dose of MDMA, and peaked 1 hour following the final dose (Figure

3.10). Further temperature recordings may have shown an even greater increase in temperature in the MDMA group.

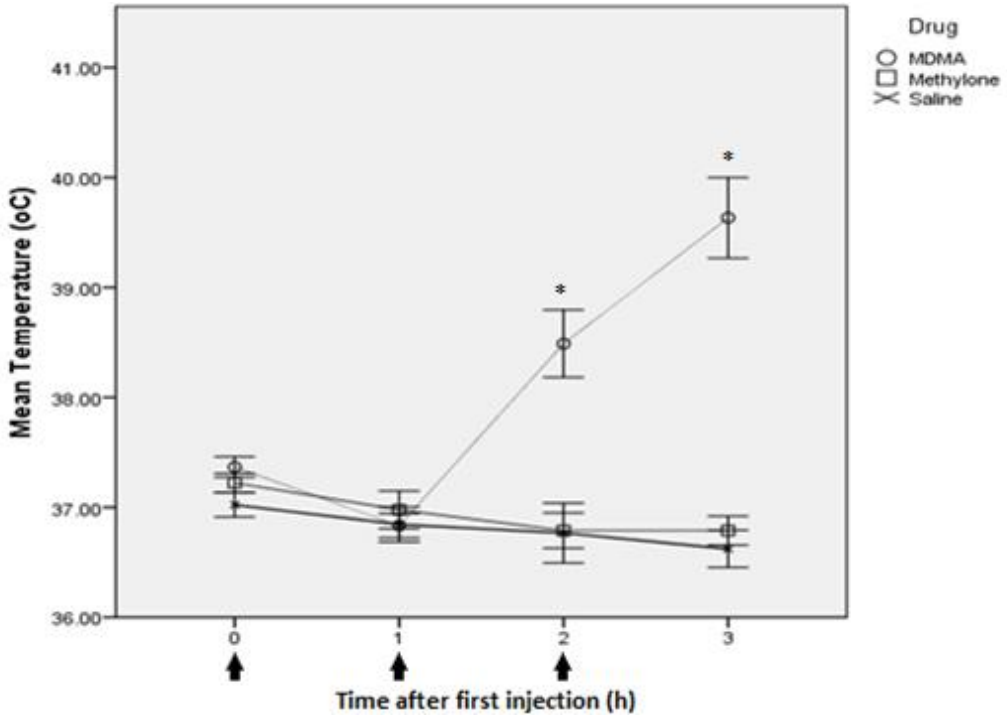


*Figure 3.10. Mean temperature of rats (°C) during repeated administration of 5mg/kg of MDMA, methylone, or equivalent volume of saline vehicle over time (h). Black arrows denote injection times. * $p < .05$ compared with saline. Error bars denote ± SEM.*

3.3 Unexpected Deaths and LD_{50}

Four of the 12 binge dosed (BD) MDMA rats (all males) died several hours after the first 15mg/kg binge dosing. They were therefore unable to be used in the following behavioural testing procedures and were excluded from statistical analysis.

The LD_{50} for male PVG/c hooded rats appears to be much lower than the LD_{50} for male Sprague-Dawley rats, which has previously been reported to be 49mg/kg (i.p.) (Hardman et al., 1973), given that 4 of 12 male rats died after 15mg/kg, and 2 of 24 male rats died after 12mg/kg

(i.p.). Based on this data, using probit maximum likelihood estimation, an LD_{50} was calculated for male PVG/c hooded rats. The iterations returned an estimated 0.5 probability of 16.14mg (CI[14.42, 28.87], $p < .05$), indicating that the LD_{50} of MDMA for male rats was much lower than previously reported.

3.4 Behavioural Testing Following Repeated Drug Exposure

Two-way factorial ANOVAs were used to compare the 5mg/kg MDMA and methylone rats and saline controls from section 3.1 (Drug Naïve, DN, group) to rats who had been given a 5mg/kg challenge of their respective drug (or saline vehicle for control rats) one week after the binge dosing regimen (Drug Treated, DT, group). Because 4 of the male MDMA rats died during binge-dosing they were not able to complete testing and were excluded from analysis.

3.4.1 Open field test.

The dependent measures in the Open Field Test were displacement, time in centre, and rearings, with drug type (saline, MDMA, Methylone), sex (M and F), and exposure (DN and DT) as the independent factors.

Locomotor Activity.

There were significant main effects for sex and drug type consistent with the results from section 3.1. However, there were no significant differences between DN and DT rats in measures of displacement (Table 3.5). Further, there were no significant drug X exposure interactions for any of the drug groups. The current results indicate that there was no evidence

for sensitisation or tolerance to either drug on the measure of locomotor activity following binge dosing.

Time in centre.

There was a significant main effect of drug type, however there were no significant main effects of sex or exposure (Table 3.5). There were no significant drug X exposure interactions for any of the drug groups. The results indicate that there was no significant effect of binge dosing of either drug on the amount of time rats spent in the centre of the open field.

Rearings.

There was a significant main effect of drug, but again there were no significant main effects of sex or exposure (Table 3.5). However, there was a significant sex X exposure interaction with both MDMA ($F(1, 56) = 4.840$, $p = 0.032$) and methylone ($F(1, 56) = 6.614$, $p = 0.013$) DT female rats demonstrating a greater number of rearings compared to DN rats, indicating a tolerance to the suppressive effect on rearing for both of these drugs. There was no significant difference in rearings for saline treated female controls ($F(1,56) = 0.423$, $p = 0.518$), indicating that this effect was not due to habituation or repeated testing. There was no significant change in number of rearings between DN and DT male rats in any of the drug groups.

Table 3.5. *F-ratios of Open Field Tests for DN and DT rats: Exposure (Between DN and DT Rats, Collapsed Across Drug Type and Sex), Drug Type (Between MDMA, Methylone, and Saline Collapsed Across Exposure and Sex), and Sex (Between M and F Rats, Collapsed Across Exposure and Drug Type).*

Open Field Tests	**Exposure**		**Drug Type**		**Sex**	
	F(1,56)	P	*F*(2,56)	P	*F*(1,56)	p
Displacement	0.858	0.358	28.18	0.000*	19.19	0.000*
Time in Centre	1.426	0.237	8.12	0.001*	0.449	0.505
Rearings	3.027	0.087	29.12	0.000*	0.598	0.442

* significant to $p < 0.01$

3.4.2. Light/Dark box.

Dependent measures for the light/dark box were the same as in section 3.1. The independent factors were sex, drug type, and exposure (DN and DT).

Time in the Light.

A two-way ANOVA showed a significant main effect for exposure ($F(1, 54) = 4.19$, $p = 0.045$). Post-hoc contrast analysis revealed that MDMA and methylone DT rats spent less time in the light side of the box than MDMA and methylone DN rats, on average ($t(40) = 2.61$, $p = .013$) (Figure 3.11). Saline controls showed no significant difference in time spent in the light between DN and DT groups, indicating that there was no effect of repeated testing or habituation. There was no main effect of drug type ($F(2, 54) = 1.73$, $p = 0.187$), however contrast analysis showed a significant difference between MDMA and methylone DT rats and saline controls ($t(32) = 2.40$, $p = 0.022$), with drug-treated rats showing significantly less time in the light. There was no main effect of sex ($F(1, 54) = 1.68$, $p = 0.206$). The data suggests

that following repeated drug exposure both MDMA and methylone rats had significantly reduced time in the light side of the LDB.

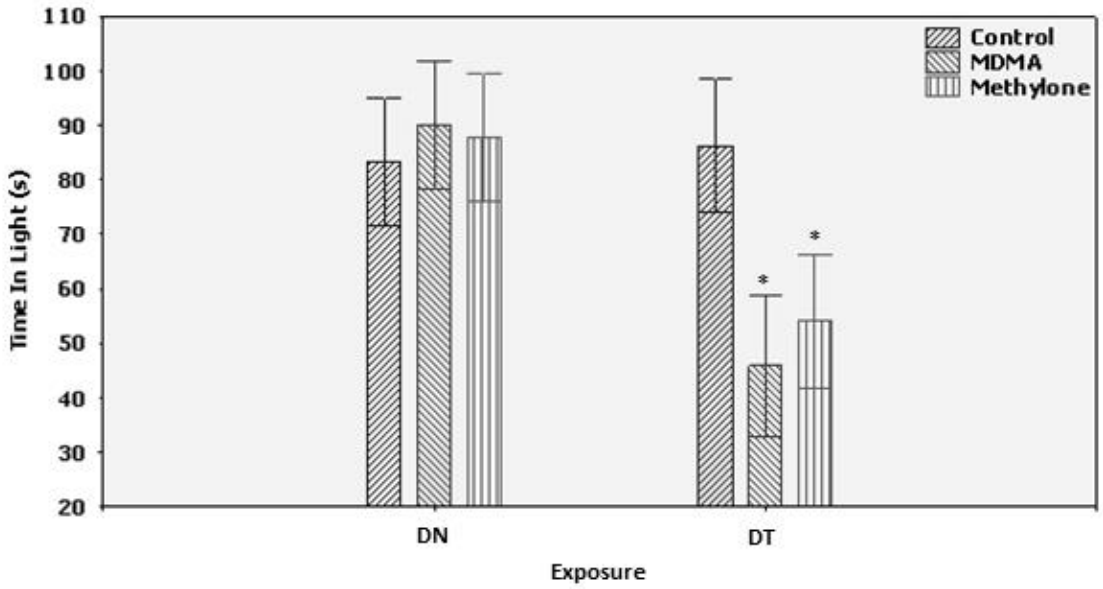


*Figure 3.11. Mean amount of time spent in the light side of the LDB for MDMA, methylone and saline treated rats by exposure to drug. DN = Drug Naïve, DT = Drug Treated. * $p < .05$ compared to control. Error bars denote ± SEM.*

Number of Transitions.

There was a significant main effect of exposure ($F(1, 54) = 5.68$, $p < .001$), with DT rats displaying fewer transitions between the light and dark sides of the box than DN rats (Figure 3.12). Again, post-hoc contrast analysis showed that this decrease was significant for MDMA and methylone groups ($t(40) = 2.76$, $p < 0.01$), with no significant difference between saline controls. There was no overall main effect of drug type ($F(2, 54) = 1.38$, $p = 0.26$). Contrast analysis showed a significant difference between drug-treated and control DN rats ($t(32) = 2.16$, $p = .038$), with drug-treated rats having a significantly higher number of transitions than controls, consistent with findings in section 3.1.2. However, following drug exposure there was no significant difference in number of transitions between drug-treated and

saline control rats. There was no main effect of sex ($F(1, 54) = 1.11$, $p = 0.296$). These results indicate that repeated exposure to MDMA and methylone attenuates the increase in transitions that was seen earlier after acute drug administration.

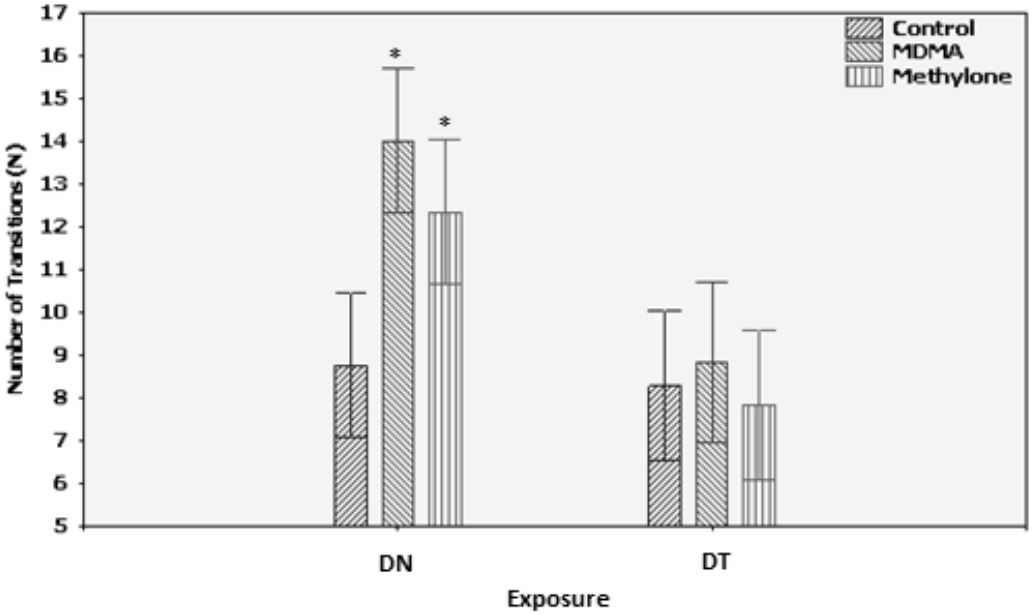


*Figure 3.12. Mean number of transitions between the light and dark compartments of the LDB for MDMA, methylone, and saline treated rats by exposure to drug. DN = Drug Naïve. DT = Drug Treated. * p < .05 compared with control. Error bars denote ± SEM.*

Emergence Latency.

There were no significant main effects of exposure, sex, or drug type, suggesting that there was no effect of repeated exposure of either drug on emergence latency. In addition, there were no significant interaction effects.

Discussion

4.1 General Summary

The current study aimed to investigate the acute behavioural effects of methylone and compare these effects to MDMA, a drug which has been extensively studied previously and has shown to be chemically and psychopharmacologically similar to methylone. Given the similarities in action on monoamine neurotransmitters, it was expected that methylone would resemble MDMA in its behavioural profile. However, because methylone has previously been shown to augment monoamine neurotransmission to a lesser degree than MDMA, it was expected that methylone would produce a behavioural response that is approximately one-half of that of MDMA.

The results demonstrated that MDMA and methylone do produce similar patterns of behaviour in many aspects, however the two drugs were not identical. In the open field, both drugs increased psychomotor activity as evidenced by enhanced horizontal locomotion, although methylone produced a greater response than MDMA, contrary to our hypothesis. This enhancement of activity was stronger in females for both MDMA and methylone treated rats, suggesting that females are more responsive to the pscyhostimulating properties of these drugs. MDMA, but not methylone, produced increased activity counts in the centre of the open field, which was surprising given several previous reports that MDMA enhanced locomotor activity in the periphery of the open field (Martinez-Price & Geyer, 2002; Rempel et al., 1993). Anxiety related behaviours were inconsistent, with MDMA and methylone reducing rearing, but having no effect on light avoidance or emergence latency and increasing the number of transitions between compartments in the LDB. In the object recognition task there was no significant effect on exploration time or object discrimination by either drug, indicating that MDMA and methylone treatment did not reduce investigatory behaviours and did not impair working memory.

The second aim of the current study was to determine the effect of a repeated binge-like exposure on the subsequent development of behavioural tolerance or sensitivity on these tests. There was no effect of previous exposure to MDMA or methylone on locomotor activity or time in the centre of the open field. For female rats, there was a significant difference in rearing activity before and after repeated drug exposure, indicating a disinhibition of the suppressing effect of MDMA and methylone on this behaviour. In the LDB there was a reduction in time spent in the light side of the compartment and reduced transitions following repeated exposure to either drug, indicating that there was more anxiogenic behaviour.

Finally, MDMA but not methylone significantly increased body temperature of male and female rats during repeated binge administration, with several fatalities in male rats. Deaths were unexpected as the dosing strategy used was similar to those used in previous studies.

These findings highlight some important similarities and differences in the acute and subacute behavioural effects of MDMA and methylone exposure. The following is a discussion of these findings, with limitations of the current study, their implications for humans, and suggestions for possible future research.

4.2 The Acute Behavioural Effects of MDMA and Methylone

MDMA and methylone are psychostimulant drugs which act on monoamine neurotransmitter by enhancing the release and blocking the reuptake of 5-HT, DA and NA (Cozzi et al., 1999). As expected, the behavioural correlates of acute MDMA and methylone exposure were similar, but not identical, and were in many aspects consistent with previous research (Gregg & Rawls, 2014).

4.2.1 Locomotor activity.

Both MDMA and methylone enhanced horizontal locomotor activity. This finding was consistent with previous research and supported the hypothesis of the current research (Callaway et al., 1991; Callaway et al., 1990). Also consistent with the hypotheses was the observation that female rats were more sensitive to the stimulant effects of these drugs. However, there were two findings which were unexpected; that methylone rats had significantly greater displacement than MDMA treated rats at higher doses, and that MDMA rats failed to display peripheral localisation of activity as seen in previous studies.

Displacement.

It was hypothesised that MDMA would have a greater effect on horizontal locomotor activity than methylone. While no studies have directly compared the behavioural effects of these two drugs previously, this hypothesis was supported by previous findings that MDMA has a greater effect on monoamine neurotransmission than methylone (Nagai et al., 2007; Sogawa et al., 2011), and that augmentation of monoamines has been shown to underlie the acute behavioural effects of psychostimulant drugs (Gudelsky & Yamamoto, 2008). Specifically, the ability of a drug to increase locomotor activity correlates with its ability to release DA in the mesoaccumbens pathway (Bubar et al., 2004). In the current study methylone was found to have a greater maximal effect on horizontal locomotor activity than MDMA, indicating that this drug causes a greater efflux of DA in this pathway.

Although methylone continued to increase ambulation as the dose increased, the hyperactivity induced by MDMA reached a maximal effect after a relatively low dose (2.5 mg/kg). This is contradictory to previous findings in other strains of rat which have demonstrated dose-dependent increases of locomotor activity up to 20 mg/kg (Kehne et al., 1996; Spanos & Yamamoto, 1989). The discordance between studies may be explained by

competing behaviours at higher doses of MDMA such as anxiety, stereotypies, or serotonin syndrome behaviours such as low body posture, all of which have previously been associated with reduced ambulation (Herin et al., 2005; Scearce-Levie et al., 1999). Indeed, higher doses of amphetamine-like stimulants have been shown to result in a phase of focused stereotypy during which locomotion declines, and this has been demonstrated in MDMA-sensitised Sprague-Dawley rats even at low doses (Ball, Klein, Plocinski, & Slack, 2011). This was the first study to use PVG/c rats, and it is possible that these rats may be more sensitive to the acute effects of MDMA, evidenced by lethality in some rats at 12 mg/kg. Thus, it is possible that at higher doses rats were developing other non-ambulatory behaviours with consequential interruption of horizontal activity.

Another possible explanation relates to the relative potencies of MDMA and methylone on various monoamine systems. The neurochemical actions of methylone have been shown to resemble MDMA, but with a reduced efficacy at increasing extracellular 5-HT levels relative to DA (Baumann et al., 2012; Cozzi et al., 1999). Previous studies have shown that 5-HT release can antagonise the stimulant and reinforcing properties of DA release seen with administration of psychostimulant drugs (Rothman & Baumann, 2006). Therefore, the higher relative effect of MDMA on 5-HT release may dampen the dopaminergic psychostimulant effects, while in methylone the reduced capacity to release 5-HT would have the opposite effect. This has important implications for the addictive potential of these drugs.

Activation of 5-HT_{1B} receptors seems to be the critical initiating event in the psychomotor activation caused by MDMA. A selective 5-HT_{1B} receptor antagonist failed to attenuate the locomotor activity caused by methylone, suggesting that this receptor does not play a role in methylone induced hyperlocomotion (Lopez-Arnau et al., 2012). Methylone may therefore be closer to amphetamines in its locomotor effects. In agreement with this, methylone has been found to be a direct agonist of 5-HT_{2A} receptors, which are known to be targets of

amphetamine compounds that mediate locomotor responses, and which may be partially responsible for its effects on locomotor activity (Lopez-Arnau et al., 2012).

Consistent with previous research, the stimulatory effects of MDMA were more pronounced in female rats (Palenicek et al., 2005; Walker et al., 2007). This was the first study to demonstrate a similar disproportionate increase in the locomotor enhancing effect in female rats for methylone. This finding is important as it implies that the acute stimulant effects of both MDMA and methylone could be higher in female human users, which could increase the risk of unwanted effects and adverse clinical outcomes. The importance of including both male and female animals in pre-clinical research is highlighted by this finding.

Central square occupancy.

MDMA has consistently been shown to increase locomotor activity in the periphery of the open field with avoidance of the centre in rats at doses up to 10 mg/kg (Palenicek 2005, Bankson and Cunningham 2002, MCreary 1999, Callaway 1990). In the current study MDMA administration dose-dependently increased time spent in the centre of the chamber, contrasting with the findings of these earlier studies.

In the open field test, anxiogenic drugs have been reported to increase time spent in the peripheral zone while decreasing exploration of the centre (Fraser et al., 2010), and it therefore could be concluded that centre avoidance seen after MDMA treatment may due to anxiogenesis. However, evidence suggests that the confinement to the periphery of the chamber after acute MDMA exposure may be due to the onset of thigmotaxis, rather than a consequence of increased anxiety. This is supported by the finding that familiarisation with the testing environment fails to change the pattern of locomotor activity, indicating that increased anxiety

is unlikely to be responsible (Callaway et al., 1991). In agreement with this, Palenicek et al. (2005) found that 10 mg/kg MDMA produced locomotor stimulation confined to the periphery of the chamber in Wistar rats, however these same rats displayed anxiolytic behaviour in the EPM, also suggesting that the confinement to the periphery was not due to anxiety (Palenicek et al., 2005).

It is unclear why the doses of MDMA used in the current study resulted in increased central activity. One explanation may be related to unrecorded observations of the MDMA treated rats which demonstrated significant focussed stereotypy of ambulation between the corner and centre in a small circular pattern, particularly in higher doses. This pattern of circular ambulation differs from that observed previously and may be due to an unusually higher sensitivity of the PVG/c rats to the stereotypic effects of MDMA. A shift from goal-directed to purposeless stereotypic locomotion has been previously demonstrated with higher doses of dopaminergic psychostimulants, and has been attributed to activity at the D_2 autoreceptor (Koulchitsky et al., 2016). It is possible that at higher doses MDMA may preferentially activate D_2 receptors, resulting in a change of motor activity from peripheral localisation to focussed stereotypies within the chamber. This theory is supported by a study by Kindlundh-Hogberg et al. (2007), who demonstrated that acute administration of high dose MDMA (3 doses 5 mg/kg doses) to male Sprague-Dawley rats significantly increased activity in the centre of an open field (Kindlundh-Hogberg et al., 2007). Therefore, MDMA may produce qualitatively different ambulation patterns depending on the dose or strain of animal used. Furthermore this change in activity may be independent of anxiety levels and is more likely due to the psychomotor stimulating and stereotypic behaviours produced by this drug.

There was no significant effect of methylone on the spatial characteristics of locomotor activity, suggesting that this drug does not produce the same characteristic patterns of stereotypic behaviour seen in MDMA. Since methylone seems to produce psychomotor

stimulation independent of 5-HT_{1B} receptors, the current findings support the notion that it is the activation of serotonergic receptors that is responsible for the spatial patterns of locomotor activity induced by MDMA (Rempel et al., 1993). Further studies using higher doses of MDMA in other breeds of rat may help to determine if the increase in central activity is a function of dose, strain, or a combination of both. Psychopharmacological studies may then prove useful to find the neurochemical basis of this behaviour.

4.2.2 Rearing activity.

It was hypothesised from previous research that MDMA would suppress rearing activity. The results confirm this hypothesis, with a dose-dependent reduction in rearing activity for all doses. It was also demonstrated that methylone significantly reduced rearing activity in a similar fashion, although the effect of MDMA on this behaviour was greater than methylone.

The suppression of exploratory activity may be due to a combination of factors including anxiety, serotonin syndrome behaviours, or decrease in exploratory drive. Firstly, it has previously been demonstrated that forced exploration in a novel environment causes elevations in "state" anxiety; that is, anxiety that is experienced in a particular moment in time and is enhanced by the presence of an anxiogenic stimulus (Belzung & Griebel, 2001; Hughes, 2007a). It has also been demonstrated that MDMA administration causes increased anxiety-like behaviour in a number of paradigms (Ferraz-de-Paula et al., 2011). Therefore the combination of MDMA and novel environment may cause high levels of anxiety in these animals, leading to a reduction in exploratory behaviour. Alternatively, it has been proposed that the reduction in rearing may be due to the onset of serotonin syndrome, characterised by low body posture, splayed hind legs, and floor sniffing, which would interfere with vertical

activity levels (Bubar et al., 2004; Palenicek et al., 2005; Spanos & Yamamoto, 1989). Indeed, in the current study rats seemed to demonstrate many of these behaviours (unrecorded observations). It is also possible that the onset of stereotypies may interrupt normal exploratory behaviour. Indeed, both a reduction in rearing activity and high levels of stereotypy were concurrently recorded after 20 mg/kg i.p. in male Wistar rats (O'Loinsigh, Boland, Kelly, & O'Boyle, 2001). Therefore, the reduction in rearing seen in the animals in the current study may be due to a combination of increased anxiety, stereotypies, and serotonin syndrome behaviours. Close examination of these behaviours in psychostimulant treated rats may help to further elucidate this distinction.

The neural mechanisms involved in MDMA-induced changes in rearing activity are not currently known, but studies have shown that it involves both serotonergic and dopaminergic mechanisms. Rempel et al. (1993) found that administration of a 5-$HT_{1B/1A}$ receptor agonist significantly decreased investigatory behaviour including rearing, and at higher doses virtually eliminated this behaviour. However, Scearce-Levie et al. (1999), found that 5-HT_{1B} knockout mice had suppression of rearing behaviour following MDMA administration similar to wild-type controls, suggesting that reductions in exploratory behaviour following administration of MDMA occurs via a mechanism other than activation of the 5-HT_{1B} receptor. In contrast, 5-HT_{1A} and 5-HT_2 agonists have been shown to reduce exploratory behaviours, while 5-$HT_{2B/2C}$ antagonists significantly potentiate MDMA induced rearing activity (Bankson & Cunningham, 2002; Mittman & Geyer, 1989; Wing et al., 1990). Bubar et al. (2004) found that MDMA (3mg/kg) consistently and robustly enhanced vertical rearing activity, and that this response was blocked by administration of D_1 or D_2 antagonists, suggesting that both D_1 and D_2 receptor activity are necessary to maintain normal rearing behaviour, as well as enhancing vertical rearing behaviour after MDMA administration. This finding indicates that normal DA activity

is necessary for exploratory behaviour, and that disruption of the dopaminergic pathways is the basis for the suppression of these activities.

It has been previously demonstrated that 5-HT_{2C} receptor mediated neurotransmission is associated with anxiogenic effects (Bagdy, Graf, Anheuer, Modos, & Kantor, 2001; Vicente & Zangrossi, 2014). The 5-HT_{2C} receptor is also known to be localised to regions containing DA cell bodies and has an inhibitory role in the control of mesolimbic DA efflux (Bankson & Cunningham, 2002). Therefore, this receptor may be responsible for the reduction in rearing activity by suppressing dopaminergic pathways in response to the neophobia towards the novel environment. Alternatively, activation of other 5-HT receptors, such as 5-HT_{1A}, may cause disruption of the DA system by activation of D_2 autoreceptors, which is known to induce stereotypic behaviour, and which could disrupt normal goal directed behaviour (Koulchitsky et al., 2016). Further studies targeting 5-HT receptor subpopulations is necessary to further characterise the neural basis of this behaviour.

Methylone was found to dose-dependently decrease rearing activity. This was consistent with the effects of MDMA, but contrary to the findings of Baumann et al. (2012) who found an increase in rearing activity after repeated administration of methylone (3 doses of 3 or 10mg/kg administered 2 h apart). However, in this study the behavioural observations were done while the rat was in its home cage, which could have removed the neophobic response that would be expected under observation in a novel environment. Therefore, methylone can cause a reduction in exploratory activity in a novel environment that may not be seen in a familiar environment. This provides evidence that methylone shares psychopharmacological effects with MDMA in this response, since both drugs produce suppression of rearing in novel environments, but may enhance rearing in familiar environments.

Taken together, these results suggest that both MDMA and methylone can cause suppression of rearing activity which may be due to activation of 5-HT_{2C} or 5-HT_{1A} receptors with subsequent disruption of normal dopaminergic activity. Since methylone produced similar results to MDMA in its effects on rearing activity, it may be inferred that the action of methylone on rearing activity is via neural mechanisms that are qualitatively similar to MDMA.

4.2.3 Light/dark box and anxiety.

The light/dark box provides a measure of unconditioned anxiety (Hascoet & Bourin, 1998). It was hypothesised that MDMA and methylone would produce anxiety-like behaviours in this test. The results showed that there was no significant difference in time that rats spent in the light side of the box, suggesting that there was no aversion to the bright light in this task, and at higher doses of both drugs it seemed that there was a trend towards increased time spent in the bright chamber. There was a significant increase in the number of transitions for both drugs, with higher doses resulting in an increased number of transitions. Finally, although there was a trend towards increased emergence latency with increasing doses of MDMA and methylone, this did not reach statistical significance.

The current study found that MDMA did not significantly affect time spent in the light side of the box, which was consistent with previous research (Maldonado & Navarro, 2000). Methylone also failed to produce any significant changes in this parameter. It has previously been suggested that an increased time in the bright chamber is associated with decreased aversion and this seems to be the most reliable parameter to assess anxiolytic activity of drugs, since this provides the most consistent dose-effect results (Hascoet & Bourin, 1998; Young & Johnson, 1991). However, the LDB test is known to produce false positive results on this parameter if the drug increases general locomotor activity (Bourin & Hascoet, 2003). In the

current study, both MDMA and methylone produced robust increases in locomotor activity. Therefore, the finding that drug treated animals did not significantly differ in the amount of time spent in the light side of the box may reflect the psychomotor stimulating properties of the drugs rather than the anxiety levels of the animals.

The number of transitions was initially thought to be an index of anxiety, however studies have not consistently shown changes to this parameter with anxiolytic drugs. This has lead researchers to believe that this parameter may be more dependent on sedative or psychostimulant properties of drugs, with decreased transitions a result of sedation (Bourin & Hascoet, 2003; Hascoet & Bourin, 1998). Transitions may be therefore be an index of activity-exploration. In the current study there was an increase in the number of transitions for both MDMA and methylone rats. This is consistent with the hypothesis that the number of transitions reflects general psychomotor activity, and like time spent in the light side of the chamber, may not be a useful measure of anxiety in psychostimulant drugs.

There was no significant change in emergence latency for either drug, which is contrary to previous findings of an increased emergence latency for MDMA (Jones et al., 2010; Morley et al., 2005), although there was a general trend towards increased emergence latency with increasing doses of either drug. In addition, five of the rats (3 MDMA and 2 methylone) failed to emerge from the dark chamber which may be a function of high anxiety levels. As with the other LDB parameters, emergence latency may have been influenced by the psychomotor stimulant properties of the drugs, and may therefore not be a reliable measure of anxiety levels in rats for these drugs.

An anxiolytic effect has been consistently reported in the EPM after moderate-high doses of MDMA, with increased entries and time spent in the open arms, which has lead researchers to suggest that MDMA may cause anxiogenesis in low doses and anxiolysis in high

doses (Lin et al., 1998). This contrasts with research using high dose MDMA in mice in the predator odour and social interaction tests which have suggested anxiogenesis at high doses (Ferraz-de-Paula et al., 2011; Maldonado & Navarro, 2001). In the LDB test, the current study found no evidence of changes in anxiety levels. There was a significant increase number of transitions, however as stated previously, this was likely to be secondary to the psychomotor stimulating or stereotypic effects of these drugs resulting in purposeless or non-goal directed ambulation throughout all areas of the testing arena (Koulchitsky et al., 2016). For this reason the EPM and LDB may be insensitive tests for the anxiogenic or anxiolytic effects of psychostimulants in animals, and previous findings of apparent anxiolytic behaviour in these tests may have also been confounded by psychomotor stimulation or stereotypies. Further studies using a wider range of tests that are not influenced by psychomotor activity may help to further understand the effects of MDMA and methylone on anxiety.

The neurochemical basis of anxiety seen after acute MDMA administration is poorly understood, with most research focussing on serotonergic mechanisms. In particular, there are a number of studies that suggest that the 5-HT_{2C} receptor is important in the expression of MDMA-induced anxiety. Hallucinogenic 5-HT_2 agonists have been shown to produce increased neophobic reactions to a novel environment which is attenuated by familiarisation (Mittman & Geyer, 1989). In addition, a selective 5-HT_{2C} receptor antagonist was shown to cause significant anxiolysis in a bright unfamiliar arena compared to saline controls, and reversed the anxiogenic effects of the SSRI antidepressants fluoxetine and sertraline in a social interaction test (Bagdy et al., 2001; Wing et al., 1990). It has also been shown that a 5-HT_{2C} receptor antagonist reduces the suppressive effect of MDMA on rearing behaviour (Bankson & Cunningham, 2002). More recently, Jones et al. (2010) found that the increased emergence latency caused by MDMA treatment in Sprague-Dawley rats was dose-dependently attenuated by a selective 5-HT_{2C} receptor antagonist. Therefore the $5HT_{2C}$ receptor may be important for

the expression of fear towards novel or aversive stimuli in MDMA and methylone treated rats. Other serotonin receptors have also been implicated in the anxiogenic effects of MDMA with less consistent results (Morley et al., 2005). However, with at least 14 different 5-HT receptor subtypes, the effect of MDMA on anxiety is likely the consequence of activation of multiple receptor subtypes in different brain regions (Lin & Parsons, 2002). More research is necessary to further classify the effects of these receptor subtypes.

It is important to remember that the topic of anxiety is complex, and the effects of MDMA and methylone on this behaviour is likely to involve multiple brain regions and neurotransmitter systems (de la Mora, Gallegos-Cari, Arizmendi-Garcia, Marcellino, & Fuxe, 2010). Previous research has demonstrated that MDMA tends to produce an anxiogenic response in most behavioural tests at low doses, while producing an anxiolytic effect in social interactions, suggesting that the pro-social effects may be mediated by different neural pathways than the effect on state anxiety (Morley et al., 2005). There are environmental factors, sex, species, and strain differences that are likely to cause differences in drug effects on anxiety (Bourin & Hascoet, 2003; Clement, Le Guisquet, Venault, Chapouthier, & Belzung, 2009). Finally, various models of anxiety are not equivalent. Models based on spontaneous responses to aversive or novel environments may produce different types of anxiety to those based on conditioning (Belzung & Griebel, 2001). All of these factors are important to consider when investigating the effects of drugs on anxiety behaviours. In the current experiment there was almost identical behaviours exhibited between MDMA and methylone treated rats suggesting similar neuropsychological effects, although the LDB may be in some ways insensitive to the effects of these drugs on anxiety, for the reasons explained above.

4.2.4 Memory.

Previous research has demonstrated that acute MDMA exposure may impair reference memory with sparing of working memory (Harper et al., 2006; Harper et al., 2005). Alternatively it may affect both working and reference memory (Braida et al., 2002). The current study used the NOR task, which is thought to be a relatively pure measure of working memory with relatively little reference memory (Harper et al., 2013). The NOR task in the current study demonstrated that rats were able to discriminate between a novel and identical object, since there was a mean discrimination ratio significantly greater than chance. There was no significant difference in object discrimination between vehicle and drug-treated rats for either MDMA or methylone. In addition, there was no significant difference in total exploration time between drug and saline treated rats, indicating that neither drug impaired exploratory drive. These results extend on previous findings and provide further evidence that MDMA does not acutely impair working memory. Furthermore, this is the first study investigating the effects of methylone on memory, and demonstrates that acute methylone treatment also does not significantly impair working memory.

While the neuropharmacology of memory deficits caused by acute MDMA administration remain largely unknown, the deficit in reference memory seems to be related to the serotonergic action of MDMA. Van Wel (2011) found that the 5-$HT_{2A/2C}$ receptor blocker ketanserin prevented MDMA induced impairment in the word learning task in human subjects, suggesting that MDMA induced impairments in verbal working memory are in large the result of direct or indirect stimulation of the 5-$HT_{2A/2C}$ receptors. However, ketanserin failed to prevent impairment of spatial or prospective memory. Using field potential recordings in rat hippocampal slices, Rozas et al. (2011) demonstrated that acute application of MDMA enhanced long term potentiation (LTP) in CA1 hippocampal neurons which involved presynaptic 5-$HT_{2A/2C}$ serotonin receptors and postsynaptic D_1/D_5 dopamine receptors,

indicating that MDMA impairs memory through a polysynaptic interaction between serotonergic and dopaminergic systems in the hippocampus. Administration of MDMA causes activation of 5-$HT_{2A/2C}$ on dopaminergic terminals, causing the release of DA which acts on D_1/D_5 receptors in the postsynaptic CA1 neurons in the hippocampus. Alterations in D_1 firing in this area causes disruptive effects on memory. The involvement of D_1 receptors was also implicated in findings by Harper (2013) who found that a D_1 receptor antagonist was able to attenuate the disruption caused by MDMA on a delayed matching to sample task in rats (Harper, 2013).

The current findings suggest that acute MDMA and methylone intoxication may not significantly impair working memory in human users, and gives weight to the suggestion that memory deficits seen in studies on humans may be secondary to non-mnemonic processes, such as attention. Future research should aim to test working and reference memory in humans while simultaneously testing for other cognitive and emotional processes and psychomotor coordination that may interfere with the testing procedure itself. In addition, the current study only addressed acute administration of these substances. Chronic self-administration of MDMA by rats has been shown to impair performance on this task when tested one week following the last administration of drug, indicating that chronic exposure may, at least temporarily, disrupt working memory (Schenk et al., 2011). Acute and chronic MDMA exposure may therefore disrupt different memory processes. Further research examining chronic MDMA and methylone exposure on neurocognitive processes may help to differentiate these effects.

4.3 Repeated Drug Exposure

The second part of the current thesis examined whether there was behavioural tolerance or sensitisation to the acute effects of MDMA or methylone after binge-type dosing. Previous research has produced mixed results with some showing sensitisation and some tolerance to the behavioural effect of this drug. The current study will build on these previous findings and is the first to determine if there is any behavioural tolerance or sensitisation following repeated exposure to methylone. Rats were dosed with 5mg/kg MDMA or methylone every hour for three hours on each of two consecutive days. One week later behaviour was tested in the open field and light/dark box following a further 5 mg/kg challenge of their respective drugs.

4.3.1 Open field test.

It was hypothesised that there would be behavioural sensitisation to the locomotor stimulating properties of MDMA and methylone following binge dosing. The results demonstrated no difference in locomotor activity for either MDMA or methylone. This is surprising given the number of previous studies that have demonstrated behavioural sensitisation following subacute dosing (Ball, Budreau, & Rebec, 2006; Ball et al., 2011; Ball et al., 2009; Bradbury et al., 2012; Kalivas et al., 1998). The differences in findings may be related to the differences in dosing regimens, the withdrawal period, or the context of exposure.

The development of tolerance or sensitisation has been shown to be dependent on whether the dose is repeated intermittent dosing or a single high dose binge (Schenk & Bradbury, 2015). Studies that have demonstrated sensitisation have generally used daily or twice daily dosing for three to five days. The current experiment used binge doses on two consecutive days which may have been an insufficient number of days to produce sensitisation. A second possibility relates to the withdrawal period. Kalivas et al. (1998) demonstrated that the sensitising effect of pre-exposure was evident in high dose binge rats after a withdrawal

period of twelve days, although after 48 hours there was no sensitisation, concluding that the sensitisation is delayed (Kalivas et al., 1998). Indeed most studies demonstrating sensitisation have done so after a delay of twelve days or longer. Therefore the use of a one week withdrawal period in the current study may not have been long enough to allow the neurocognitive changes necessary for augmentation of the effects of MDMA to occur. In contrast, a study by Ball et al. (2006) found sensitisation in locomotor activity after a three to five day withdrawal period, but only in rats who received their sensitising doses in the apparatus used for behavioural testing rather than in the home cage. This finding of "dependence on context of exposure" has been consistently replicated, and suggests that the consequential development of sensitisation to MDMA is dependent on the context in which the drug is given, particularly following short withdrawal periods (Ball et al., 2011; Ball et al., 2009). In the current study the drug was given in the home cage which could have reduced the effect of sensitisation. Future research on tolerance and sensitisation to the effects of psychostimulants should be aware of these procedural manipulations and the effect they could have on behavioural outcomes.

The underlying mechanisms of the augmented locomotor response to MDMA are not clearly known, and may be due to repeated effects on DA neurotransmission or via altered 5-HT receptor mechanisms (Schenk & Bradbury, 2015). McGregor et al. (2003) found that a two day binge of MDMA could alter 5-HT receptor density 3 months after exposure, with high dose exposure causing an increase in 5-HT_{1B} receptor density in the NAc, but low dose causing a decrease in 5-HT_{1B} density in other brain regions. Given the importance of these receptors in the locomotor response to MDMA, this differential response could at least partly account for why different dosing regimens can lead to different outcomes. Alternatively, the increased sensitivity to MDMA after repeated administration may be a consequence of structural changes to the DA system through neuroplasticity (Schenk, 2011). Ball et al. (2009) found that intermittent binge dosing of MDMA for three weeks can cause reorganisation of synaptic

connectivity in the limbic-cortico-striatal circuitry, with increases in dendritic spine density in the NAc. More recently, Lettfuss et al. (2013) have proposed that behavioural sensitisation may be mediated by muscarinic receptors (Lettfuss, Seeger-Armbruster, & von Ameln-Mayerhofer, 2013). More research is necessary to determine what underlying changes occur from repeated MDMA and methylone exposure, and what experimental manipulations may enhance or reduce this effect.

Unfortunately the current experiment failed to replicate earlier findings of behavioural sensitisation in locomotor activity following acute MDMA exposure. This may have been due to the strain of rat used or due to the experimental procedures used as previously mentioned. It remains unknown whether methylone is capable of producing behavioural sensitisation or tolerance after repeated administration. Further research on this topic is recommended.

4.3.2 Rearing activity.

Previous studies have demonstrated that repeated MDMA exposure can lead to an increase in rearing activity, possibly due to behavioural sensitisation to the psychostimulatory effects (Lettfuss et al., 2013; Schenk & Bradbury, 2015). In the current study there was a significant effect of exposure on rearing activity for female rats, with both MDMA and methylone binge exposure causing an attenuation of the drug-induced suppression of rearing activity. There was no effect of pre-exposure on rearing by male rats for either drug. Therefore it seems that female rats are more susceptible to behavioural sensitisation than male rats, which is likely related to their higher sensitivity to the stimulatory effects of the psychostimulants. This is consistent with previous reports demonstrating higher sensitisation of female rats to the pscyhostimulating effects of MDMA following repeated exposure (Walker et al., 2007).

Alternatively, the disinhibition of rearing activity seen in female rats following repeated exposure may be due to reduced stereotypic or serotonin syndrome behaviours. The most

frequently documented neurochemical change following repeated administration is 5-HT depletion (Green et al., 2003; Jones et al., 2010). It seems reasonable to suggest that this would cause a reduction in serotonin syndrome or stereotypic behaviours, which may lead to an increase in goal-oriented exploratory behaviours. Therefore the increased rearing activity seen in female rats may be a combination of 5-HT depletion causing tolerance to the serotonergic effects, and neuroplastic changes in DA neurotransmission in the NAc causing sensitisation to the stimulant effects (Schenk, 2011).

These findings indicate that greater neuroplastic or neurotransmitter changes occur in females from the subacute exposure to MDMA, which could mean that they are at greater risk of long term psychological and neurocognitive sequelae from drug use. For this reason the importance of including both male and female participants in studies on psychostimulant drugs is emphasised.

4.3.3 Light/dark box test

The previous literature regarding tolerance or sensitisation to the anxiogenic effects of MDMA after repeated administration is sparse, but suggests that there may be development of tolerance possibly due to the 5-HT depleting effects of MDMA (Bull et al., 2004; Jones et al., 2010). The current study found that there was a significant reduction in time spent in the light side of a light/dark box and a significant reduction in the number of transitions after pre-exposure to MDMA or methylone. This effect was unlikely to be due to a habituation or repeated testing effect since saline controls showed no differences between trials. There was no effect of pre-exposure on emergence latency, contrary to the findings by Jones et al. (2010), suggesting that there was no change in baseline anxiety levels.

Following exposure to MDMA and methylone there was a significant reduction in time spent in the light side of the light/dark box, which contrasts with the acute effects in drug naïve

animals as previously described. Pre-exposure had the opposite effect on transitions, reducing the previously seen increase in transitions after acute exposure back to the level of saline treated control rats. After acute exposure it was argued that the failure of these drugs to attenuate time spent in the light side of the chamber or transitions may have been due to the psychostimulant action of these drugs, resulting in non-goal directed ambulation about all areas of the light/dark box. Thus, the drug effects on anxiety would not be detected using these parameters since they are confounded by psychomotor behaviour (Bourin & Hascoet, 2003). However, following binge dosing it is possible that there was a tolerance to the stereotypic behaviour produced by these drugs, and the reduction in time spent in the light side of the box may actually be a result of the anxiogenic properties of these drugs. Therefore, while there was no tolerance or sensitisation to the quantity of ambulation as seen in the open filed, it is possible that there was a change in the quality of locomotion with less stereotypical behaviours, possibly via reduction in dopaminergic D_2 receptor stimulation (Koulchitsky et al., 2016). A closer examination of stereotypic behaviour caused by MDMA and methylone may have provided further support to this theory, and should be taken into consideration in future studies examining the behavioural effects of these psychostimulant drugs. Overall, previous exposure to both drugs was able to alter the subsequent behaviour in the light/dark box, with a significant reduction in time spent in the light, and attenuation of the drugs effect on transitions, indicative of anxiogenesis.

These findings support the idea that both MDMA and methylone cause anxiety in low doses, but do not provide any evidence for a tolerance to this anxiogenic response following repeated drug exposure, since there was no change in emergence latency. The reasons for the lack of tolerance seen in the current study may be for similar reasons as the failure to demonstrate sensitisation to the locomotor effects, explained previously. Whether or not these drugs can cause tolerance in their anxiogenic effects remains largely unknown. Given the importance of anxiety in the development of drug abuse and dependence, further research

should be conducted to determine if there is a reduction in this effect from chronic drug exposure.

4.4 Temperature, Toxicity, and Rat Strain

Temperature changes in the acute administration of amphetamine derivatives is important as acute hyperthermia has been closely correlated to the degree of serotonergic neurotoxicity caused by MDMA in the rat, although the ability of MDMA to cause neurotoxicity in humans is controversial (Docherty & Green, 2010; O'Loinsigh et al., 2001). Even so, hyperthermia has also been thought to play a crucial role in MDMA lethality (Green et al., 2003; Koenig et al., 2005).

Previous studies have demonstrated hyperthermia for both MDMA and methylone following acute exposure to these drugs (Baumann et al., 2012; den Hollander et al., 2013; Green et al., 2003). The current study found that MDMA caused a significant and marked rise in temperature with repeated administration, but there was no effect on temperature for methylone. The mean rise in temperature for MDMA was 2.3^{o}C and peaked at three hours after the first dose of MDMA, and it is possible that it would have continued to increase further if recording had continued. The hyperthermic effect seen in the current study is similar to those previously reported in male Sprague-Dawley, Dark Agouti, and Wistar rats (Green et al., 2003). There was no significant rise in temperature for methylone treated rats, which is contrary to previous findings. The reason for this may be related to the doses used in the current experiment which were much lower than in previous studies (Baumann et al., 2012; den Hollander et al., 2013). Regardless, the current study demonstrates that the effect of MDMA on hyperthermia is much more prominent that the effect of methylone, and may therefore have a much greater risk in terms of toxicity.

There were six unexpected fatalities from 12 to 15mg/kg MDMA in the current study, all occurring in male rats. This was surprising given that previous studies have used comparable doses of MDMA in other male rat strains, including Sprague-Dawley and Wistar rats, without fatalities (Kalivas et al., 1998; O'Loinsigh et al., 2001). In addition, the LD_{50} for male rats was calculated using probit maximum likely hood estimation which gave an approximate LD_{50} of 16.14 mg/kg (i.p.), which is much lower than the 49 mg/kg (i.p.) which has previously been reported for male Sprague-Dawley rats (Hardman et al., 1973). PVG/c hooded rats used in the current study may therefore be more susceptible to the acute toxic effects of MDMA.

Alternatively, the high lethality of MDMA seen in these rats may be partially explained by aggregation toxicity. Ho et al. (2004) injected group-housed male Wistar rats with 15 mg/kg i.p. which resulted in fatality in 14 of the 17 rats. The authors concluded that the high fatality rate seen may have at least partly been due to these rats being group-housed during acute drug administration, given that O'Loinsigh et al. (2001) administered a higher dose of 20 mg/kg i.p. in this same rat strain in singly housed rats with no fatalities (Ho et al., 2004). Indeed, both social interaction and high ambient temperature, conditions that mimic those in which humans often consume MDMA, have been found to potentiate the vasoconstricitve effects and fatality in Long Evans rats (Kiyatkin et al., 2014). The current study group housed rats during the binge dosing procedure, and this may have contributed to the high rate of fatalities seen. This finding is important in terms of human drug use, as users of MDMA often do so in close social environments and often seek closer contact with others due to the drugs enactogenic effects. This may enhance the subjective effects as well as the toxicity of the drug.

Male humans and rodents may be more sensitive than females to the acute toxic effects and hyperthermia related fatalities from MDMA (Fonsart et al., 2008; Koenig et al., 2005). The current findings support this hypothesis. Therefore, while the acute behavioural psychostimulant effects are more pronounced in female rats, the acute toxic effects of MDMA

are greater in males. This finding has important implications for male human users of MDMA, and may partly explain why there was a 4:1 (male/female) sex ratio in the number of fatalities associated with this drug previously reported (Schifano, 2004). This sexual dimorphism again highlights the importance in using both male and female animals in studying the effects of drugs.

4.5. Implications of the Current Findings

4.5.1 Addiction

The potency of a drug to block the DAT or to enhance dopaminergic neurotransmission is associated with its psychostimulant effect and abuse liability (Rothman & Baumann, 2003, 2006). Alternatively, drugs that increase 5-HT are not abused and increased 5-HT relative to DA activity may actually reduce the addictive potential of the drug (Rothman & Baumann, 2006; Wee et al., 2005). It has been found that, in MDMA self-administration paradigms, MDMA is a weak-to-moderate reinforcer with only a subset of rats acquiring self-administration (Bradbury et al., 2014; Cole & Sumnall, 2003b). Rats that fail to acquire self-administration tend to have greater 5-HT overflow, suggesting that 5-HT may limit the positively reinforcing effects of MDMA (Bradbury et al., 2014). Alternatively, increased locomotor activity may directly relate to DA activity in the NAc, and therefore the reinforcing and addictive properties of psychostimulant drugs (Bubar et al., 2004; Gatch et al., 2013).

Previous studies have shown that methylone produces elevations in DA and 5-HT quantitatively similar to MDMA, but with a diminished capacity to release 5-HT relative to DA and reduced overall potency (Baumann et al., 2012; Simmler et al., 2013). The finding that methylone produced greater locomotor activity than MDMA confirms that this drug has greater

relative action on dopaminergic neurotransmission, and may therefore have a higher abuse potential. In agreement with this, previous studies have found that methylone produces dose-dependent IV self-administration through spontaneous acquisition procedures, and appears to produce more robust self-administration acquisition than comparable studies using MDMA (Nguyen, Grant, Creehan, Vandewater, & Taffe, 2016; Schenk et al., 2007; Watterson et al., 2012). In addition, escalation of methylone intake in extended accesses self-administration was greater than that for rats trained to self-administer MDMA, demonstrating higher abuse potential (Nguyen et al., 2016; Vandewater, Creehan, & Taffe, 2015). However, self-administration of methylone increased less than cocaine and methamphetamine, suggesting that the reinforcing properties of methylone are weaker, and that the potential for compulsive use in humans is less likely, than these primarily dopaminergic psychostimulants (Nguyen et al., 2016; Watterson et al., 2012).

4.5.2 Safety and toxicity

The current study demonstrated that MDMA can produce fatalities in male rats in doses as low as 12 mg/kg. However, allometric scaling of effective and toxic doses of MDMA from animals to humans is complex since differences in metabolism and formation of toxic metabolites differ among animal species (de la Torre & Farre, 2004). In addition, the route of administration in the current study (i.p.) is different to that of human users (oral), which has been shown to dramatically affect the plasma concentrations and production of toxic metabolites (Baumann et al., 2009). Finally, the context in which the drug is taken appears to be important for its toxic potential, given that aggregation toxicity has been previously demonstrated (Kiyatkin et al., 2014). Therefore, although previous studies have attempted to translate toxic and neurotoxic doses in rats or mice to humans, such estimates are probably inaccurate (Green, King, Shortall, & Fone, 2012), which places greater importance on human pre-clinical studies. What can be concluded from the current study is that MDMA is more toxic

and may produce greater neurotoxicity than methylone at equivalent doses in this breed of rat, given the hyperthermic response and fatalities produced by MDMA. Whether this translates to human users is unknown. Rats may be a reasonable model for examining the neurotoxic effects of MDMA since it is known to produce serotonin depletion consistent with findings in humans and other primates (Green et al., 2003).

It is important to remember that the amount of methylone or MDMA in tablets bought on the streets vary widely, and many of these pills are likely to contain multiple psychoactive chemicals (Brunt et al., 2016). A recent study in the UK found that the mean amount of MDMA in one tablet was close to 60mg. However, there was wide variability, with a bimodal distribution of content between 20-40 mg and 60-80mg (Wood et al., 2011). This disparity in drug concentrations emphasises the importance of the potential harms associated with "ecstasy" use, and the need for more vigorous drug testing of street drugs in order to provide safety information and education to the public, and to track which other chemicals are being found in these illicit drugs.

4.5.3 Sex differences

The current findings are consistent with previous reports that MDMA produces greater psychomotor effects in females (Palenicek et al., 2005; Walker et al., 2007), and extends the literature with evidence that methylone also has a greater stimulant effect on females. While there was no difference between males and females in the reduction in rearing activity following acute drug exposure, there was an attenuation of MDMA and methylone suppressed rearing in female rats following binge dosing. This may be due to increased behavioural sensitisation to the drugs in female rats, or due to the reduction in stereotypic or serotonin syndrome related behaviours following binge exposure. Enhanced sensitisation in female but not male rats following repeated MDMA exposure has been previously demonstrated (Walker

et al., 2007). This means that female rats may have a higher degree of neuroplastic changes following drug exposure.

Amphetamine-induced psychopathology has been related to the progressive sensitisation of locomotor effects following repeated exposure (Kalivas et al., 1998). Therefore, the findings of enhanced stimulant effects and sensitisation for females is important as it may mean that human female users of MDMA and methylone may experience greater acute and chronic adverse neuropsychiatric effects, especially since females tend to weigh less than males but consume the same size tablets (Palenicek et al., 2005). Indeed, women have been reported to show stronger responses to MDMA in the clinical setting, with significantly higher ratings for both positive and negative effects (Liechti et al., 2001). Even though no clinical studies have been performed on humans using methylone, it is expected that there would be a similar pattern of sex differences from the current findings.

On the other hand, male rats were more sensitive to the acute toxic effects of MDMA, with lethality at 12 and 15mg/kg. Therefore, although females may be more sensitive to the psychostimulant properties of MDMA, males may be more at risk of acute toxicity and death. This may partly explain the higher incidence of death reported in male users of MDMA (Schifano, 2004).

These findings highlight the importance of including both male and female animals in pre-clinical studies of drugs of abuse, particularly given the current predominance of male bias in neuroscience and behavioural pharmacological research and the consistent findings of sex related differences in the effects of drugs (Beery & Zucker, 2011; Hughes, 2007b).

4.5.4 Anxiety

The current research found evidence of an anxiogenic response to the acute administration of both MDMA and methylone. While there was no significant difference in time spent in the light side of the box and an increase in transitions in drug-naïve rats, there was a significant reduction in time spent in the light side of the box and attenuation of the number of transitions after binge-dosing. This may be interpreted in one of two ways; either MDMA and methylone only produced increased anxiety after repeated exposure, or MDMA and methylone also produced anxiety after the initial acute exposure, but expression of this response in the LDB was confounded by the psychomotor and stereotypic behaviours induced by these drugs. The second explanation seems more feasible, given that numerous previous studies have demonstrated anxiogenic effects from both acute and chronic MDMA exposure. Thus, it appears from the current findings that both MDMA and methylone exposure can produce anxiety-like behaviour. This is consistent with findings in studies using human participants, who have been shown to score higher on indices of anxiety (Kuypers, Wingen, & Ramaekers, 2008), and suggests that methylone may have a similar subjective effect on anxiety in human users. This is important given the number of emergency department admission for panic attacks and anxiety related behaviours such as paranoia that have been reported in the literature after consumption of MDMA (Liechti, Kunz, & Kupferschmidt, 2005), and suggests that methylone may carry an equivalent public health liability in this regard.

4.5.5 Memory and cognitive problems

It has been previously reported that acute MDMA administration may cause a temporary impairment in working and visuospatial memory in humans (Kuypers & Ramaekers, 2005, 2007; Kuypers et al., 2008), although non-mnemonic causes for these deficits cannot be ruled out. Previous studies in animals have suggested that the memory impairments seen in the

eight-arm radial maze may be due to impaired reference memory with relative preservation of working memory (Harper, 2013; Harper et al., 2006; Kay et al., 2010). The current study failed to demonstrate any impairment in working memory for MDMA and methylone using the NOR task, even at high doses of either drug that would be largely in excess of doses typically used by humans, and therefore supports the idea that the deficits in memory seen in animal studies may be due to a specific impairment in reference memory. Deficits in working memory seen with acute intoxication in humans may therefore be a function of a more global deficit in neurocognitive functioning or due to non-mnemonic factors that have not been accounted for, rather than a specific working memory impairment.

4.6 Limitations of the Current Study

There were several limitations to the current study worth mentioning. Firstly, the doses of MDMA used in this breed of rat was too high, given that there were several deaths. This meant that the number of rats for each conditions was reduced, particularly in the binge-dosing experiments, with a reduction in statistical power. This was the first study to use PVG/c hooded rats in behavioural studies using MDMA and methylone. The doses used were based on previous similar studies using other rat strains (Kindlundh-Hogberg et al., 2007; McCreary et al., 1999; Rodsiri et al., 2011), and it was unanticipated that this strain of rat would be more susceptible to the acute toxic effects of MDMA. This highlights the importance of differences between strains and species of animal in their pharmacokinetics and metabolism of drugs. Lab animals often receive doses of drugs which are much higher than those taken recreationally by humans and by routes of administration that are not typical of human consumption (Baumann 2008). While allometric scaling is difficult, it is clear that the MDMA dose of 12mg/kg is far higher than that used by recreational users since it caused substantial lethality.

The binge dosing procedure used in the current experiment was unable to cause behavioural sensitisation that has been previously observed. This may have been due to the short binge dosing period used, which was only two days. Previous research has demonstrated that three to five days of daily or twice daily dosing is generally required in order to produce behavioural sensitisation (Schenk & Bradbury, 2015). Alternatively it may have been due to the short latency period between the last dose and behavioural testing. Behavioural sensitisation has been shown to generally take more than twelve days to develop following the last dose (Kalivas et al., 1998), while in the current study behavioural testing occurred after one week. It is possible that if we had waited for two weeks we may have seen more behavioural changes following binge dosing. Future research should take these parameters into consideration when designing tests for tolerance and sensitisation to psychostimulants.

Another limitation is that the behavioural tests for anxiety, including time in the centre of the open field, rearing activity, and the LDB, may have been confounded by the psychomotor stimulation or stereotypic behaviours produced by each of these drugs. In the LDB the number of transitions has traditionally been attributed to changes in anxiety (Bourin & Hascoet, 2003), while in the current study the increase in transitions may have been due to a general increase in locomotor activity. Time spent in the light side of the box and central ambulation in the open field could both be influenced by the onset of stereotypic, or non-goal directed, behaviours which have been demonstrated previously in MDMA treated rats (O'Loinsigh et al., 2001). In addition, rearing behaviour may have been reduced by serotonin syndrome behaviours such as low body posture as previously suggested (Palenicek et al., 2005; Spanos & Yamamoto, 1989). Thus it would have been beneficial to measure the stereotypic and serotonin syndrome behaviours for both MDMA and methylone so that they could be accounted for when interpreting this behavioural data. Secondly, since psychomotor stimulation and stereotypic ambulation could confound the results in the LDB and EPM these tests of anxiety may not be

appropriate for MDMA and methylone, and this may be the reason why previous studies have found conflicting results in terms of anxiolysis or anxiogenesis for MDMA in high doses (Ferraz-de-Paula et al., 2011; Lin et al., 1998). Future research using these drugs should carefully measure stereotypic behaviours in order to determine whether observed behaviours are truly due to the cognitive processes that they intend to measure, or whether they are confounded by the onset of aimless repetitive behaviours.

Finally, there is a lot of individual variability in the response to MDMA in human users, particularly at higher doses (Baylen & Rosenberg, 2006; Downing, 1986; Harris et al., 2002). This was also evident in the current study since the standard error in observations increased proportionately with increasing doses of both MDMA and methylone. This implies that the behaviour of the rats became less predictable at higher doses, which may have been a function of individual idiosyncratic differences between animals, and reduced the power to make statistically significant findings. It may be possible to stratify animals based on prior behaviours in order to predict individual traits, and therefore account for this when performing statistical analysis. For example, Ludwig et al. (2008) divided Wistar rats into high anxiety or low anxiety sub-groups based on their behaviour in an EPM screening test, and found that behavioural sensitisation and reduction in anxiety was more pronounced for low anxiety rats following multiple daily injections of MDMA (Ludwig, Mihov, & Schwarting, 2008). Thus identification and consideration of individual differences in rats may allow researchers to make more accurate predictions of subsequent behaviour.

4.7 Future Research

There are several important considerations from the current study that should be addressed in future research. To begin with, the current study only looked at the acute

behavioural effects and development of tolerance or sensitisation from subacute dosing of MDMA and methylone. With increasing widespread abuse of psychostimulant drugs, more research is needed investigating the acute and chronic neurocognitive effects of these drugs in humans and animals using a wider range of cognitive tasks. Thus, future studies should also look at the chronic effects of repeated administration on areas of neurocognition, such as memory, and neuropsychology, such as anxiety. Impairment in memory and development of chronic anxiety has been previously attributed to repeated MDMA exposure, so determining whether this is also seen in chronic methylone exposure warrants further investigation.

The current research used PVG/c rats which have not previously been used in acute behavioural studies using MDMA. The doses used were consistent with previous research but led to a high number of fatalities. Using a consistent strain of rat for drug studies, such as Sprague-Dawley which has predominantly been used in previous research on MDMA, allows easier interpretation and comparison between studies. However, this may also lead to a rather facile view of the effects of these drugs. Indeed, the current study illustrated that the LD50 of MDMA may be strain dependent, and could therefore be more unpredictable and dangerous in human users than previously anticipated. In addition, there has been a predominance of using only male animals in neurobiological research (Beery & Zucker, 2011; Hughes, 2007b). The higher sensitivity of female rats to the acute effects of psychostimulants, and the higher toxicity seen in males, provides further evidence that sex bias in research jeopardises our understanding of sexual dimorphism in the effects of drugs.

The measurement of anxiety levels in the current study was difficult since the development of psychomotor stimulation and stereotypic and serotonin syndrome-like behaviour confounded interpretation of the results. Traditionally, exploratory behaviour, time spent in the aversive light side of the LDB, number of transitions, and emergence latency have all been associated with changes in the levels of anxiety in mice and rats (Bourin & Hascoet,

2003; Jones et al., 2010). However, the use of these parameters in the assessment of the anxiogenic or anxiolytic effects of MDMA and methylone may not be reliable since they are confounded by the general psychomotor effects of these drugs. Previous studies using the EPM have demonstrated increased time in the open arms with high doses of MDMA which has been interpreted as anxiolysis (Ferraz-de-Paula et al., 2011; Ho et al., 2004; Palenicek et al., 2005), however whether these result were confounded by the same opposing behaviours as seen here is not known. This illustrates the importance of choosing behavioural tests wisely, while taking note of confounding behaviours, in order to maximise internal validity.

From pharmacological studies alone it was predicted that methylone would have a behavioural response that would be approximately half that of MDMA. What was observed, however, was that methylone produced dose-dependent increases in locomotor activity that were greater than those observed with MDMA. This highlights the importance of conducting both neurochemical and behavioural studies in order to draw appropriate conclusions about the effects of drugs. In addition, the doses used in the current study were too high and caused multiple fatalities. Future research should aim to use doses that appropriately scale to typical human users in order to improve face validity. Correlation of the dose-response obtained in different strains and species of animal to the dose-response obtained in human pre-clinical studies may help in this regard. However, the legal and ethical restraints of using controlled substances in human subjects inhibits such progress.

Further research investigating the nature of DA and 5-HT interactions will help our understanding of the complex interplay between these systems. The use of psychostimulants are a valuable research tool that allow us to augment neural systems and carefully observe the behaviours produced, which can then be correlated with the psychopharmacological effects. The use of monoaminergic drugs such as MDMA and methylone are important in this regard. For example, the onset of stereotypies by these drugs may provide useful clues to the neural

mechanisms that underlie conditions characterised by an excessive tendency to repetition, such as Tourette syndrome and obsessive-compulsive disorders, which are thought to be caused by abnormal dopaminergic activity (Ford, 1991).

4.8 Conclusion

Methylone is an interesting new designer psychostimulant with similar psychopharmacological and behavioural effects to MDMA. The current study is the first to directly compare behaviour after acute and subacute administration of MDMA and methylone in rats. We were able to show that MDMA and methylone shared similar but distinct behaviours in a wide range of tests. Specifically, we were able to show that methylone has greater psychostimulant effects than MDMA, and therefore seems to demonstrate a cocaine-MDMA-mixed behavioural profile as previously anticipated from pharmacological studies (Simmler et al., 2013). This has important implications in terms of the abuse liability for methylone, and supports the current enforcement of control of this substance. In addition, we demonstrated that female rats were more susceptible to the acute stimulant effects of both drugs, while male rats were more sensitive to the acute toxic effects of MDMA. Thus, drugs of abuse demonstrate sex related differences which may have important consequences when extrapolating animal data to humans. Data concerning the chronic effects of MDMA and methylone are lacking, and this warrants further research.

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