THE EFFECTS OF ACUTE AND BINGE 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) EXPOSURE ON LEARNING AND MEMORY IN RATS.

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ABSTRACT

When rats are administered acute doses of MDMA they produce significantly more reference memory errors than working memory errors in the partially baited radial arm maze (Kay et al, 2009). The potential role of serotonin and dopamine in this effect was examined by administering the serotonin agonist Citalopram and the dopamine agonist GBR12909. GBR12909 produced significantly more reference memory errors, while Citalopram tended to produce more working memory errors. Administration of the D_1 agonist A68930 and the D_2 agonist Quinpirole predominantly produced reference memory errors, but to a lesser extent than acute MDMA administration. Low doses of both drugs produced a synergistic effect, more similar to that seen with acute MDMA administration. These findings suggest dopamine plays a role in the reference memory effect seen with MDMA exposure in the partially baited radial maze.

In the second half of the thesis binge regimes of MDMA (4 x 10mg/kg) were administered to rats. When there was a gap of eight weeks between dosing and training the ability to acquire the radial arm maze was not significantly impaired. When this MDMA regime was repeated with a three-day gap between dosing and training it produced a significant but transient deficit in performance. When later challenged with acute doses of MDMA (4.0 mg/kg) the binge treated rats were less impaired than saline controls indicating drug tolerance. In an additional study that used a three-day delay between dosing and training a significant impairment in task acquisition was found. This deficit appeared to be long-term as the MDMA treated rats were impaired when the rules of task were changed suggesting a deficit in cognitive flexibility. Again

when subjects were challenged with acute MDMA there was evidence of drug tolerance. The final study examined the effects of repeated MDMA exposure on task acquisition by administering acute doses of MDMA or saline once a week after rats had previously been treated with either a binge regime of MDMA or saline. MDMA exposure significantly impaired task acquisition and produced residual drug effects in the binge treated MDMA group the day after acute drug administration. However evidence of behavioural tolerance in this study was mixed due to a floor effect where performance of the binge MDMA group was so poor at the beginning of the study.

In conclusion MDMA exposure impaired accuracy with reference memory processes were more affected than working memory processes. The underlying nature of this impairment remains unclear but it may be due to a long-term memory deficit, an impairment in understanding task rules or a perseverative pattern of responding. These findings imply human Ecstasy users may show deficits in acquiring information and may experience deficits in cognitive flexibility

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GENERAL INTRODUCTION

What Is Ecstasy/MDMA?

Ecstasy or 3,4-methylenedioxymethaphemtamine (MDMA) is a ring substituted amphetamine structurally similar to methamphetamine (Farre et al., 2004). Its street names include XTC, E, or X (Cottler, Womack, Compton & Ben-Abdallah, 2001) and it is chemically related to hallucinogens and stimulants (Peroutka, Newman & Harris, 1988). It has been described as producing a unique state of euphoria and heightened self-awareness (Peroutka et al., 1988). In addition it increases self confidence, intimacy, depth of emotion and sensory awareness (Morgan, 2000). However, it does not produce the psychotic effects and hallucinations associated with other hallucinogenic drugs (Peroutka et al., 1988). MDMA is both a serotonin and dopamine agonist and it is unclear which neurotransmitter system is responsible for the positive drug effects reported by Ecstasy users (Parrott, 2002).

The recreational use of Ecstasy increased dramatically during the late 1990s (Wilkins, Bhatta, Pledger & Casswell, 2003). This trend has received a lot of media attention with reports of severe toxicity and fatalities producing widespread concern (Morgan, 1999) and has been recognised as a major public health issue around the world (Kish, 2002). There is debate as to whether Ecstasy is a relatively benign substance (von Sydow, Lieb, Pfister, Hofler & Wittchen, 2002) and even a valuable treatment for a range of psychological conditions (Grob, 2000). Most researchers have reported Ecstasy users experiencing a number of unpleasant side effects including psychological and cognitive problems (Morgan, 2000; Parrott 2001). Others have

suggested it may be a harmful neurotoxin (McCann, Szabo, Scheffel, Dannals & Ricaurte, 1998).

A Brief History: How MDMA Became Ecstasy

MDMA has been reported as an appetite suppressant; but was never originally designed or used for this purpose (Freudenmann, Oxler & Bernschneider-Reif, 2006). It was patented in Germany in 1912 by the pharmaceutical company Merck as a precursor chemical for other therapeutic compounds (Freudenmann et al., 2006). During the 1950s in the USA it was researched for its toxicity and potential as a 'brainwashing' weapon (Eisner, 1994). It resurfaced during the mid 1970s where therapists in the USA and Switzerland used it in psychotherapy (Holland, 2001a). However by the early 1980s MDMA started to be used recreationally as an illicit substance (Hatzidimitrious, McCann & Ricaurte, 1999) and acquired the name Ecstasy (Holland, 2001b). As recreational use increased, the USA Drug Enforcement Administration (DEA) held emergency hearings arguing MDMA caused brain damage (Grob, 2000) based on animal research indicating MDA (a drug related to MDMA) produced brain damage in rats (Holland, 2001b). Although therapists argued MDMA had clinical uses this was based on anecdotal evidence lacking the necessary double-blind, placebo controlled studies (Holland, 2001b). Therefore in 1985 MDMA was classified as a Schedule 1 drug due to its potential for abuse and lack of medical use. In 1986 the World Health Organisation and the United National Commission on Narcotic Drugs classified MDMA as a Schedule 1 drug internationally and it is illegal in many other countries around the world including Australia, Canada, United Kingdom and New Zealand (Holland, 2001c).

Despite the drug being illegal MDMA consumption has become widespread with Ecstasy use increasing over 4000 percent between 1990 and 1995 in the United Kingdom alone (Holland, 2001a). There are several studies indicating Ecstasy use is still increasing in Europe and the USA (Daumann, Fimm, Willmes, Thron & Gouzoulis-Mayfrank, 2003) as well as Australia (Lyvers, 2006). While there have been many studies that have found Ecstasy users tend to be young, white and from middle class families there are reports that users are becoming more diverse (Bahora, Sterk & Elifson, 2009). There is clear evidence that Ecstasy use is increasing and it appears there is a range of different demographic, social and psychological variables that contribute to Ecstasy use.

Why Is Ecstasy/MDMA Important To Study?

Many young people are taking the drug and the medical, social and psychological consequences of this are unclear. One issue is the conflicting information about Ecstasy found in both the media and the scientific realm. For example, Green (2004) proposed there has been a lot of erroneous reporting by the media about the dangers of MDMA. Green (2004) argued that the claims made about the dangerousness of MDMA are often inaccurate and increased knowledge is necessary in both the scientific and popular press. Some claims made by the media relating to the dangers of Ecstasy have not been critically evaluated and have been reported in order to scare people away from using Ecstasy which may weaken the credibility of research findings (Lyvers, 2006). Obtaining accurate information that can be delivered to the public is of particular importance to parents. Ecstasy use is the least discussed drug between parents and teenagers (Alcoholism and Drug Abuse Weekly, 2003). A reason for this is that parents do not know the effects of the drug and are

unable to recognise whether their children are under the influence of the Ecstasy (Alcoholism and Drug Abuse Weekly, 2003).

Also of concern is the finding Ecstasy use tends to be associated with potentially other risky health behaviours such as binge drinking, polydrug use and a larger number of sexual partners (Strote et al., 2002; Boyd et al., 2003). However, due to the mainly cross sectional nature of prevalence studies it is unclear as to the direction and cause of these behaviours. Similarly several studies have found Ecstasy use has been associated with elevated impulsivity (Morgan, 1998; McGuire, 2000; Morgan, McFie, Fleetwood & Robinson, 2002; Butler & Montgomery, 2004) and impairments in the ability to accurately judge reinforcement cues producing impaired decision making (Morgan, Impallomeni, Pirona & Rogers, 2006). Morgan et al. (2006) argued these impairments may produce problems in everyday functioning and may contribute to continued drug use. However, it is difficult to conclude Ecstasy use produces increases in impulsivity as drug takers may be naturally more impulsive and more likely to take drugs in the first place (Morgan, 1998). Elevated impulsivity and risk taking are associated with other drugs of abuse and since Ecstasy users tend to be polydrug users it may be these other substances that are contributing to elevated impulsivity (Butler & Montgomery, 2004).

Another important issue is that many Ecstasy users appear to perceive the harm of Ecstasy use as low and many believe it to be a safe drug (Green, Cross & Goodwin, 1995) that produces few health problems (Bahora et al., 2009). Ecstasy users also believe the drug is not addictive (Bahora et al., 2009) and has a low potential for abuse (Ball, Walsh & Rebec, 2007). A reason for this could be the lack of craving and withdrawal effects generally experienced by users (Bahora et al., 2009). However, there are reports of tolerance developing to Ecstasy as the positive effects decrease over time with repeated ingestion (Parrott, 2001).

Is Ecstasy/MDMA Harmful?

Ecstasy or MDMA has been used as a therapeutic drug by psychotherapists in the 1970s and 1980s (Liester, Grob, Bravo & Walsh, 1992). During the 1980s the drug was administered to human volunteers and researchers concluded Ecstasy was reasonably safe, produced positive changes in mood and did not appear to have negative consequences (Downing, 1986). Positive effects reported by Ecstasy users include euphoria, enhanced feelings of well-being, an increase in desire for social contact and more energy (Hegadoren et al., 1999).

However, there do seem to be a lot of negative side effects associated with Ecstasy use. Some of the commonly reported acute effects of Ecstasy ingestion include tachycardia (rapid heart rate), dry mouth, tremors, palpitations, diaphoresis (excessive sweating), parasthesias (skin sensation, such as burning, prickling, itching, or tingling) (Peroutka et al., 1988). Sub-acute effects that have occurred 24 hours after Ecstasy ingestion included drowsiness, aching muscles, fatigue, depression, trismus (jaw clenching), difficulty concentrating, headaches, anxiety and irritability (Peroutka, et al., 1988). Other commonly reported side effects include mydriasis (dilated pupils), photophobia (light sensitivity), decreased appetite, nausea, abdominal cramps, diarrhoea, sweating, tachypnea (rapid breathing), bruxism (teeth grinding), and ataxia (difficulty walking) (Henry & Rella, 2001). Less reported but more severe side effects include hallucinations, severe anxiety, agitation, panic attacks, paranoia, hypertension, cardiac arrhythmias, chest pain, severe abdominal cramps and urinary retention (Henry & Rella, 2001). There are also more delayed side effects which can include jaundice, hepatotoxicity, tooth wear, poor concentration and attention, memory impairment, depression, sleep disturbance, weight loss and exhaustion (Henry & Rella, 2001).

There have been reports of fatalities related to Ecstasy use. However, most of the data relating to Ecstasy fatalities comes from case reports or small case series making it difficult to accurately generalise to large populations. Hence estimates of the risk of using Ecstasy vary from one death in 2000 to one death in 50000 (Schifano, 2004). However compared to the number of people who use the drug the number of deaths attributed to Ecstasy use is low (Klys et al., 2007). Most of the deaths related to Ecstasy use have been attributed to hyperthermia, hyponatremia and 5-HT syndrome.

A problem with the reported side effects and fatalities is that they have only been associated with MDMA use and we cannot infer causality. Many studies involving fatalities do not test for the presence of MDMA (Kish, 2002). Another problem is Ecstasy tablets are often cut with other psychoactive substances (Green et al., 1995). Polydrug use is also an important factor as Ecstasy users often abuse other illicit drugs (Morgan, 2000). This makes it difficult to ascertain if these problems are due to MDMA, other drugs, or some interaction between different substances. Finally there is the issue that Ecstasy users tend to have poor lifestyles and engage in behaviours that may affect their health, such as irregular patterns of sleep and food intake (Parrott, 2000).

Ecstasy and Neurotoxicity

While the evidence regarding potential negative health and behavioural effects of MDMA from studies of human users is equivocal evidence has accumulated from animal studies since the mid 1980s that MDMA can produce major alterations in the serotonergic system in the brain (Grob, 2000). Animal studies suggest MDMA causes elevation of the neurotransmitter serotonin (5-hydroxytryptamine, or 5-HT) (Kish, 2002). Normally 5-HT is tightly regulated within the brain; however MDMA floods

the synapse with abnormally high amounts (Marlberg & Bronson, 2001). MDMA is unusual pharmacologically as its effects on the serotonergic system are two-fold in that it not only releases 5-HT from the pre-synaptic neuron but it also inhibits 5-HT reuptake (Marlberg & Bonson, 2001). Within four hours of acute MDMA administration there can be an 80% loss of 5-HT within the brain (Green, Cross & Goodwin, 1995). In fact three to six hours after ingestion such a large amount of 5-HT has been released that it causes temporary 5-HT depletion (Marlberg & Bonson, 2001). In addition MDMA temporarily inactivates the enzyme tryptophan hydroxylase that is required to make 5-HT resulting in the brain being unable to make enough 5-HT to restore levels to normal (Marlberg & Bonson, 2001). Normally the ability to synthesise 5-HT returns within 24 hours of Ecstasy ingestion (Baumann, Wang & Rothman, 2007) and therefore normal levels can be restored (Marlberg & Bronson, 2001). However prolonged high doses of MDMA have been associated with long-term 5-HT depletion (Marlberg & Bonson, 2001). This is of importance as 5-HT plays an important role in various bodily functions such as mood modulation (Roiser & Sahakian, 2004) and low levels of 5-HT are correlated with depression (Marlberg &Bonson, 2001).

MDMA also releases the neurotransmitters dopamine and norepinephrine but to a lesser extent than 5-HT (Kish, 2002). These neurotransmitters tend to be less studied in MDMA research (Colado, O'Shea, & Green, 2004). However, Colado et al. (2004) argued the dopamine release found after MDMA administration may play a significant role in the behaviours associated with taking MDMA such as alterations in mental state. They further argued more attention needs to be focussed on the chronic and acute effects of MDMA on dopamine (Colado et al., 2004). Other researchers have also reported that it is unclear as to whether some of the effects of Ecstasy use are due to the drugs affects on the serotonergic or the dopaminergic systems (Parrott, 2002).

One of the most important and as yet unanswered questions is whether MDMA produces neurotoxicity within the human brain (Peroutka et al., 1988). Researchers examine neurotoxicity by examining the effect of regimes of MDMA that usually involve repeated large doses of the drug, in non-human animals (Baggott & Mendelson, 2001). Studies using this approach with rats have found these regimes produced prolonged reductions in the concentration of brain 5-HT and its metabolite 5-HIAA, the number of 5-HT uptake sites and the action of tryptophan hydroxylase (Ricaurte, 1989). Anatomical studies have also found evidence that indicate damage to serotonergic axons (Ricaurte, 1989). These effects have also been found in a number of other species including guinea pigs, monkeys and baboons (Ricaurte et al., 2000).

The serotonergic damage can last for months or even years after exposure to the drug (Ricaurte, Yuan, & McCann, 2000) and has been found in several brain regions such as the hippocampus, striatum, neocortex and thalamus. Hatzidimitrious et al, (1999) found chronic doses of MDMA in squirrel monkeys produced 5-HT damage seven years after drug ingestion suggesting damage maybe permanent. Of particular concern is primates are 4 to 8 times more sensitive than rodents to the neurotoxic effects of MDMA that has led researchers to argue humans may also show greater sensitivity (Ricaurte, 1989).

Unfortunately human studies have been unable to conclusively verify whether the brain damage seen in animals occurs in Ecstasy users. One problem is that human studies rely on more indirect measures of neurotoxicity. One method involves measuring levels of the 5-HT break-down product 5-HIAA in cerebrospinal fluid (Reneman, Booij, Majoie, van den Brink & den Heeten, 2001). While some studies have found cerebrospinal fluid levels of 5-HIAA were lower in Ecstasy users compared to non users there have also been some that have found no differences

(Baggott & Mendelson, 2001). In addition there is controversy about the accuracy of these measures (Steele, McCann & Ricaurte, 1994).

Other evidence of neurotoxicity in humans comes from scanning techniques such as positron emission tomography (PET). One of the first Ecstasy studies to use PET scans was conducted by McCann et al. (1998) who found a reduction in brain 5-HT transporter binding in previous Ecstasy users compared to the controls and greater Ecstasy use produced larger reductions. McCann et al. (1998) argued this was evidence Ecstasy users are at risk of 5-HT brain damage. Other studies have also utilised PET scan technology and found evidence of altered 5-HT activity in Ecstasy users (Obrocki et al., 1999).

In addition MRI imaging techniques have shown evidence of axonal injury in Ecstasy users and this damage was positively correlated with the extent of previous Ecstasy use (Reneman et al., 2001). Functional magnetic resonance imaging (fMRI) also found a trend for heavy users to have weaker activation in the left frontal and temporal areas of the brain. Daumann et al. (2003) suggested this may be indicative of subtle differences in brain functioning due to Ecstasy use.

Again there is controversy as to how well these methods assess damage and polydrug use and the purity of Ecstasy are issues that make it difficult to assess whether the damage seen is due to MDMA or other substances (Reneman et al., 2001). Due to these methodological limitations no one technique has been able to conclusively answer whether MDMA causes neurotoxicity in humans. However, there is converging evidence suggesting MDMA can damage 5-HT neurons in the human brain. Particularly, those who take higher doses and use the drug longer may be vulnerable to MDMA induced brain damage.

Recently there has been controversy as to whether MDMA can be described as a neurotoxin. There is evidence that MDMA does not destroy neurons but rather damages the axons leaving the nucleus intact (Baumann et al., 2007) and that 5-HT terminals are not destroyed as the damage seen may be eventually reversed (Baumann et al., 2007). In addition MDMA exposure may simply deplete 5-HT to levels that are undetectable rather than damaging the neurons (Baumann et al., 2007). In fact there has been research suggesting MDMA should not be labelled a neurotoxin as there are drugs that produce similar effects to MDMA but are not categorised neurotoxic (Baumann et al., 2007).

In addition the validity of animal research has been questioned based on the amount of MDMA administered to animals. The doses given to laboratory animals tend to comprise of multiple or single doses of 10 to 20 mg/kg whereas the average human recreational dose tends to be between 1 to 3 mg/kg (Baumann et al., 2007). The route of drug administration used in animal studies has also been questioned as animals are often injected with MDMA while humans take the drug orally (McKenna & Peroutka, 1990). There has also been debate about the frequency of drug administration used in animal research. Animals are often given MDMA twice a day for four consecutive days (Ricaurte, Yuan & McCann, 2000). This research may be valuable as it suggests Ecstasy users who go on "binges" may be more susceptible to damage (Baggott & Mendelson, 2001) but it has been criticised as the majority of users tend to take the drug once a week (Morgan, 2000). However, there is evidence that patterns of use are changing as users are taking the drug more frequently (Parrott, 2002).

Perhaps the most relevant issue is whether the MDMA induced 5-HT damage actually produces corresponding behavioural disturbances (Grob, 2000). 5-HT is involved in many functions including learning and memory and hence it would be

expected that 5-HT depletion should result in deficits in these areas (Grob, 2000). However the evidence for this is mixed. An explanation for this is a large amount of 5-HT depletion may be needed for noticeable physical symptoms to appear. Large doses of MDMA produce about 40 to 60% 5-HT depletion in most brain regions (Bauman et al., 2007) which may not be sufficient to produce noticeable behavioural symptoms. For example visible symptoms in patients with Parkinson's disease only occur when 80-90% of the dopamine neuronal pathway is depleted (Grob, 2000). Therefore a large amount of neurotransmitter depletion may be necessary for noticeable behavioural changes to be detected. In addition it has been suggested that some cognitive tests have not been sensitive enough to detect impairments after MDMA exposure (Baumann et al., 2007).

Recently a study that claimed MDMA produced dopamine neurotoxicity was retracted as the rats had accidentally been administered methamphetamine rather than MDMA (Ricaurte, Yuan, Hatzidimitrious, Cord & McCann, 2003). Subsequent studies revealed no evidence of MDMA producing dopamine neurotoxicity (Ricaurte et al., 2003). This has brought many of the claims that MDMA produces neurotoxicity into question. Indeed Lyvers (2006) argued the haste with which this article was published highlights the eagerness of researchers and governments to report findings indicating the dangerousness of Ecstasy.

Cognitive Problems Associated With Ecstasy Users

Because 5-HT has been implicated in normal learning and memory (Ricaurte et al., 1993) a number of studies have examined the effects of Ecstasy use on cognition. Of particular concern is some of the brain areas (hippocampus and cerebral cortex) found to suffer 5-HT damage due to Ecstasy use are strongly associated with memory

function (Parrott, 2000). Therefore, Ecstasy users may suffer various cognitive impairments. Indeed Ecstasy use has been associated with general mental confusion (Davison & Parrott, 1997) and significant impairments on a variety of cognitive tasks (Heffernan, Ling & Scholey, 2001). Within the field of cognition there are many different types of memory function. This is important as drugs may impair certain memory functions while leaving others intact.

While Ecstasy users have shown more general memory deficits (Rodgers, 2000) there are also more specific areas of cognitive function that are impaired with Ecstasy use. One area of memory function that seems to be impaired in Ecstasy users is the recall of verbal information (Heffernan et al., 2001). Tasks that assess verbal learning and recall of verbal stimuli have found Ecstasy users required more trials than controls to reach the same level of performance (Fox, Toplis, Turner & Parrott, 2001; Gouzoulis-Mayfrank et al., 2000). Other researchers have also found impaired verbal learning in Ecstasy users (Reneman et al., 2001; McCardle, Luebbers, Carter & Croft, 2004). Ecstasy users also show significant deficits in the immediate and delayed recall of verbal material indicating they have problems with retrieving verbal information (Bolla, McCann & Ricaurte, 1998; Parrott & Lasky, 1998; Reneman et al., 2000; Rodgers, 2000; Fox et al., 2001; Verkes et al., 2001; Reneman et al., 2001; Morgan et al., 2002; Gouzoulis-Mayfrank et al., 2003; Dafters, Hoshi & Talbot, 2004; McCardle et al., 2004; Smith, Tivarus, Campbell, Hillier & Beversdorf, 2006). As Ecstasy users produce impaired delayed recall they show impairments in encoding information into long-term memory (McCardle et al., 2004) and retrieving learnt information from long-term memory (Fox et al., 2001). In addition the degree of memory impairment has been correlated with evidence of 5-HT damage in Ecstasy users (Reneman et al., 2000).

One area of the brain affected by MDMA exposure is the forebrain, including the frontal cortex (Heffernan, Jarvis, Rodgers, Scholey & Ling, 2001). This brain region is thought to be important in executive functioning (Heffernan et al., 2001) which involves controlling many higher cognitive functions such as monitoring and updating working memory, inhibition, task-shifting, planning, concept formation and cognitive flexibility (Roesch-Ely et al., 2005; Dafters, 2006). Zakzanis and Young (2001) found impaired executive functioning in abstinent Ecstasy users using an extensive battery of tests that assessed time estimation, planning, problem solving and rule learning. In addition the more Ecstasy subjects had used, the more prominent these impairments were. Wareing, Fisk and Murphy (2000) assessed executive functioning in Ecstasy users utilising a random-letter generation task. Ecstasy users found the task very difficult and were impaired on several measures of the task with some participants failing to complete it.

Verbal fluency measures have also been impaired in Ecstasy users (Croft, Mackay, Mills & Gruzelier, 2001; Gouzoulis-Mayfrank et al., 2000; Montgomery, Fisk, Newcombe & Murphy, 2005) suggesting they have executive functioning impairments as well as deficits in accessing long-term memory (Montgomery et al., 2005). Ecstasy users also have produced significant impairments on the Tower of London (TOL) suggesting strategic planning deficits (Fox, Parrott & Turner, 2001). Interestingly Ecstasy users who did not report problems associated with their drug use produced impaired performance implying they were unaware of their impairments (Fox et al., 2001).

Ecstasy users have also been more susceptible to interference effects (Gouzoulis-Mayfrank, 2000), associative learning deficits (Croft et al., 2001), cognitive flexibility, task switching impairments (Von Geusau, Stalenhoef, Huizinga, Snel & Rudderinkhof, 2004; Dafters, 2006) and a tendency to perseverate (Von

Geusau et al., 2004; Montgomery et al., 2005). While these studies have examined the effects of long-term use, Smith et al. (2006) examined the transient or acute effects of Ecstasy. They found 10 to 15 hours after drug ingestion participants were impaired on tasks that assessed executive functioning including measures of rule learning, cognitive flexibility and problem solving.

Other researchers have not found such convincing evidence that Ecstasy users are impaired at tasks assessing executive functioning. Fox, McLean, Turner, Parrott, Rogers and Sahakian (2002) administered an array of tests measuring working memory, verbal fluency, attention, associative learning and decision making to Ecstasy users. Ecstasy users were only impaired on one task assessing executive functioning which measured verbal fluency. They also found Ecstasy users had short-term memory deficits. Similarly Gouzoulis-Mayfrank et al., (2003) found no significant deficits on tests assessing working memory, executive functioning, impulsivity and planning in Ecstasy users. However they did find Ecstasy users had impaired recall. Gouzoulis-Mayfrank et al. (2003) therefore argued Ecstasy users suffer from memory disturbances rather than executive dysfunction and as these deficits are subtle they may not be detected resulting in further drug taking exposing users to an increased risk of further cognitive deficits (Gouzoulis-Mayfrank et al., 2003).

One commonly studied executive functioning process is working memory which entails the capacity to temporarily store and manipulate information (Howard et al., 2003). Gouzoulis-Mayfrank et al. (2000) reported that while the specific cognitive deficits associated with Ecstasy use remain ambiguous it appears that working memory in particular seems to be affected by Ecstasy consumption. This is supported by the large number of studies that found Ecstasy users are impaired on tasks that assess working memory processes (Croft et al., 2001; Fox et al., 2001; Verkes et al., 2001;

Morgan et al., 2002; Jacobsen, Mencl, Pugh, Skudlarski & Krystal, 2004; Von Geusau et al., 2004; Wareing et al., 2004).

Some researchers have argued that laboratory studies have failed to inform us about the memory functioning of Ecstasy users in a more natural context. Therefore, other types of memory function have also been examined. Prospective memory functioning involves remembering to do something in the future (Heffernan et al., 2001). Ecstasy users show impairments on tasks assessing prospective memory (Heffernan, Ling & Scholey, 2001; Heffernan, Jarvis, Rodgers, Scholey & Ling, 2001) and both current and former Ecstasy users have also shown evidence of impaired visuospatial memory (Wareing, Murphy & Fisk, 2004).

One potential confound within the research that examines the cognitive impairments found in Ecstasy users is that they tend to be polydrug users and there is some debate as to whether it is the Ecstasy that produces the cognitive deficits or other drugs they have ingested. For example Croft et al. (2001) found cognitive impairments in Ecstasy users who also used cannabis and Ecstasy free cannabis users. There were no significant differences between the two groups on the degree of cognitive impairment they produced so Croft et al. (2001) argued cannabis use is an important confound in Ecstasy research. Rodgers (2000) and Dafters et al. (2004) also found Ecstasy and cannabis users and cannabis only users both showed significant memory impairments suggesting the deficits found in the Ecstasy users could be due to cannabis use rather than Ecstasy use.

However other researchers that have controlled for polydrug use have found supporting evidence that Ecstasy use is the contributing factor to the cognitive impairments found in Ecstasy users. Morgan (1999) found Ecstasy users were significantly impaired on tests assessing memory compared to Ecstasy free polydrug

users and drug free controls. Other researchers have also found that the memory impairments found in Ecstasy users remained significant when other drug use, including cannabis, were taken into account (Heffernan et al., 2001; McCardle et al., 2004; Wareing et al., 2004). Gouzoulis-Mayfrank et al. (2000) and Dafters (2006) found participants who used Ecstasy and cannabis were significantly impaired on several cognitive measures but participants who used only cannabis did not show these impairments. Therefore there is evidence that Ecstasy use and not polydrug use is associated with cognitive deficits.

There is also some concern as to whether memory deficits in Ecstasy users remain after they have stopped using the drug. Some studies have examined Ecstasy users that have abstained from drug use for a short periods, usually one to two weeks, and found they still show impairments in cognitive tasks (Bolla et al., 1998; Gouzoulis-Mayfrank et al., 2000; Fox et al., 2001; Verkes et al., 2001; Zakzanis & Young, 2001; Fox et al., 2002; Gouzoulis-Mayfrank et al., 2003; Dafters et al., 2004; Von Geusau et al., 2004; Montgomery et al., 2005). However these studies do not inform us about the long-term consequences of Ecstasy use. There has been evidence that Ecstasy users who have abstained for longer periods such as two to four months (Rodgers, 2000; Reneman et al., 2000; McCardle et al., 2004) and even up to six months (Wareing et al., 2000; Wareing et al., 2004) still show significant deficits on a number of cognitive tasks. In addition there have been reports of Ecstasy users showing cognitive impairments after abstaining from Ecstasy use for several years (Reneman et al., 2001; Morgan et al., 2002) suggesting with long-term drug cessation the deficits in memory function may not subside.

In conclusion there is extensive evidence that Ecstasy users experience a wide range of impairments on cognitive tasks. Because there has been such a range of impairments it is difficult to identify what mechanism might underlie these deficits.

There are also some methodological limitations with many of these studies. For example human studies are less controlled than animal research due to legal and ethical restrictions that only allow the study of the chronic use of the drug by recreational users (Curran & Travil, 1997). Therefore, they are unable to utilise traditional double-blind placebo-controlled drug studies that would help infer causality (Curran & Travil, 1997). Another problem is Ecstasy users are commonly polydrug users and therefore any impairment seen could be the result of ingesting other substances (Morgan, 2000). In addition Ecstasy tablets commonly contain substances other than MDMA making it difficult to determine if any impairment seen is due to other chemicals found in the tablets (Green et al., 1995). Another concern with these studies is they are cross sectional in design and do not provide a measure of memory performance before participants began using Ecstasy. This is problematic as we do not know if people had cognitive problems beforehand.

There is also a paucity of research on the acute effects of MDMA. It would be useful to know what kind of impairments people experience while under the influence of the drug. Obviously due to legal and ethical issues acute studies on human participants are unlikely. Unfortunately, all of these factors make it difficult to claim MDMA actually causes cognitive deficits in humans. Animal models are beneficial as they provide a more controlled environment to study the effects of MDMA on cognition. Therefore, the current thesis will focus on animal research involving both the acute and chronic/binge effects of MDMA on cognition.

The Current Thesis

The thesis is divided into two parts that will focus on the acute and chronic/binge effects of MDMA on learning and memory in rats. The first part of the

thesis will examine the acute effects of Ecstasy on memory performance utilising a radial arm maze. It will include a review of the animal literature examining the acute effects of Ecstasy on learning and memory. Specifically it will focus on examining which neurotransmitter systems may be responsible for the memory deficits produced by acute MDMA exposure. The second part of the thesis will focus on the binge effects of MDMA on learning in rats. This is an attempt to model some of the long-term effects of Ecstasy use on memory and learning in humans. By administering a binge regime of MDMA to rats the long-term effects of Ecstasy use can be mimicked. By comparing the performance of MDMA treated rats with saline controls it can be ascertained if MDMA produces learning deficits that will be displayed by an impaired ability to acquire a radial arm maze task.

PART ONE:

Acute Effects of MDMA on Non-human Animals

Acute MDMA Human Studies

Few studies have examined the acute effects of MDMA on human participants. One group of studies was conducted by researchers in Switzerland. Human volunteers given MDMA reported alterations in feelings of time and space with participants feeling dreamy or lost in thought (Vollenweider, Gamma, Liechti & Huber, 1998). Also at higher doses (1.80 mg/kg) MDMA produced thought disturbances including impaired decision-making and losing track of one's thoughts (Liechti et al., 2001). Vollenweider, Liechti, Gamma, Greer and Geyer (2002) summarised a series of experiments that examined the acute effects of MDMA on human volunteers in Zurich. Drug naive participants in placebo-controlled double blind experiments were administered a single oral dose (1.35-1.8 mg/kg) of MDMA. Various physiological and psychological measures were used as well as PET scans. They also examined information processing by examining pre-pulse inhibition (PPI) which assesses the ability to filter out cognitive or sensory stimuli (Vollenweider et al., 2002). MDMA exposure produced an increase in positive mood, well-being, emotional sensitivity and mild disturbances in thinking such as difficulty concentrating and making decisions. However it did not produce hallucinations and PET scans revealed a change in activity in cortical, limbic and paralimbic areas of the brain. In conclusion acute administration of MDMA in healthy drug naive volunteers produced a number of physiological and psychological effects such as mood enhancement and changes in the brain structures

associated with emotion as well as altering cognition by affecting the ability to filter out stimuli (Vollenweider et al., 2002).

To date no study has utilised placebo-controlled double blind experiments with drug naive participants to specifically examine the acute effects of MDMA on memory and learning tasks. Instead previous or current MDMA users have served as participants. Kuypers and Ramaekers (2007) investigated the effects of a single dose of MDMA on spatial memory with recreational MDMA users. They utilised a double-blind, placebo controlled design to examine the acute effects of the drug on memory 1.5 to 2 hours post administration while participants were under the influence of the drug. They also assessed participants during the withdrawal phase 25.5 to 26 hours after administration. Acute administration of MDMA produced significantly worse performance on a less demanding spatial memory task. However, this impairment was not present during the withdrawal phase indicating the effect of the drug was short-term. Performance on a more complex change blindness task was not significantly affected during either phase. Therefore, Kuypers and Ramakers (2007) argued acute MDMA exposure affects spatial memory while more complex contextual processing is spared. Although Kuypers and Ramaekers (2007) tried to control for confounding variables by using double blind placebo control measures and using predominantly light Ecstasy users this type of study is still problematic in that it did not use naive drug users. In fact some of the subjects in the Kuypers et al. (2007) study were classified as heavy users that had taken MDMA on 60 to 120 occasions. Therefore, this study could not control for the effects of long-term drug use on performance.

Acute Non-human Animal Studies

Much of the previous research on the effects of MDMA has focused on chronic treatments of MDMA that try to mimic the long-term effects of the drug. However, a few studies have tried to assess the effects of acute MDMA exposure on cognition. These paradigms generally involve smaller doses of the drug being administered and the subjects' performance on various tasks is measured while the drug is present in the animals system. Studies that have examined the acute effects of MDMA on memory have produced mixed results with some finding evidence of MDMA producing cognitive deficits while others have not.

For example Byrne, Baker and Poling (2000) examined the chronic and acute effects of MDMA on acquisition of a lever pressing task. Water deprived Sprague-Dawley rats were administered intraperitoneal injections of 0.0 (saline), 1.0, 3.2 or 5.6 mg/kg of MDMA. The task involved placing rats in an operant chamber with two levers, one lever produced reinforcement (water delivery) and the other if pressed cancelled the schedule reinforcer. MDMA exposure increased the latency of the rats to start responding but MDMA did not reduce the overall number of reinforcer responses or produce any impairment in discrimination learning between the reinforcer and cancellation levers.

DMTS/DNMTS Tasks

Rather than examining learning by studying task acquisition, more commonly research has focussed on what the effects of MDMA exposure are on tasks that assess memory. One of the most commonly used tasks to assess memory function in non-human animals are delayed matching to sample tasks (DMTS) (Edhouse & White, 1988). These are conditional discrimination procedures where subjects have to match

various stimuli that are separated in time by a range of delays (Edhouse & White, 1988). The procedure begins with the presentation of a sample stimulus which is removed for a delay period after which various stimuli are presented concurrently (White, Ruske & Colombo, 1996). In a DMTS task the correct response would be to pick the stimulus that was identical to the sample stimulus (White et al., 1996). There are variations of this paradigm called delayed non matching-to-sample tasks (DNMTS) and in these tasks the correct response would be to pick the stimulus that was different to the sample stimulus (Dudchenko, 2004). The terms delayed matching-to-position (DMTP) and delayed nonmatching-to-position (DNMTP) are sometimes used when the task involves stimuli that vary in terms of their location rather than stimuli that differ in visual characteristics. DMTS tasks have been argued to assess memory by modelling human episodic recognition tasks (Harper et al., 2005) and are commonly used to assess short-term or working memory (White et al., 1996).

Using these tasks two different patterns of impairment can be found. The first is referred to as delay independent as overall performance is disrupted across all delays (Harper et al., 2005). This type of impairment has been argued to be the result of attention or encoding deficits (Harper et al., 2005). Delay dependent impairments occur when performance is worse at longer delays than shorter delays (Herremans, Hijzen, Olivier & Slangen, 1995). This type of impairment indicates an impairment in working memory (Herremans et al., 1995) or an increase in the rate of forgetting (White et al., 1996).

LeSage, Clark and Poling (1993) examined the effects of acute doses of MDMA in pigeons using a DMTS task with three different delays (0, 3 & 6 seconds). Doses ranged from 0 to 5.6 mg/kg of MDMA. MDMA decreased accuracy and response rate in a dose dependent fashion at all delays. Harper, Wisnewski, Hunt and Schenk (2005) also examined the effect of MDMA on DMTS performance in rats. They administered

acute doses of MDMA (0.3 to 3.0 mg/kg) and found it produced a delay-independent decrease in performance that increased with drug dose. Hence acute MDMA appears to produce deficits in encoding or attention (Harper et al., 2005). Utilising a DNMTS task Marston, Reid, Lawrence, Oliverman and Butcher (1999) examined the effects of administering large ascending doses (10, 15 & 20 mg/kg) of MDMA to rats. MDMA exposure disrupted performance in trials using longer delays and also produced significantly more bias (Marston et al., 1999). These delay-dependent impairments in performance were attributed to the acute MDMA administration disturbing short-term memory (Marston et al., 1999).

However, not all studies that have examined the effects of acute MDMA exposure on DMTS tasks have found evidence of impairment. For example Frederick, Gillam, Allen and Paule (1995) and Frederick and Paule (1997) both utilised a battery of operant tasks that included a DMTS task to examine the acute effects of MDMA administration on Rhesus monkey. Both studies found no significant differences in performance between saline and MDMA (0.1-1.0 mg/kg) administration. Of note both Frederick et al. (1995) and Frederick and Paule (1997) used smaller doses of MDMA than most other studies which may account for the lack of deficit.

There is some debate about which cognitive processes DMTS and DNMTS tasks actually assess. These tasks have typically been used as evidence of working memory deficits. Working memory is a temporary memory that is trial dependent as it is only relevant for one trial (Santin et al., 2003). It involves the rat being able to hold in memory where it has been and where it has yet to go within a trial. Harper et al. (2005) found acute MDMA exposure produced an overall delay independent impairment in accuracy that is usually considered to represent a deficit in working memory probably due to an attentional deficit. However, Harper et al. (2005) offer an alternative

explanation in that these tasks actually involve a reference memory component and this may be what is disrupted in DMTS tasks.

Reference memory is trial independent as the information available for performing tasks requiring reference memory is constant from trial to trial (Santin et al., 2003). Reference memory is used to learn the general rules or strategies required to solve the task and refers to the stable elements of stimulus control related to the task (Harper et al., 2005). It is trial independent as it does not matter what trial you are in as the rules of the task remain the same. In terms of the DMTS task the reference memory component could involve responding to a sample and then choosing to respond to the comparison stimuli that matched the sample. Therefore, Harper et al. (2005) argued there are other elements of stimulus control at work in DMTS tasks and it is possible a rat could become impaired or confused as to the rules of task.

Instead of simply looking at discrimination or accuracy Harper et al. (2005) performed further analyses on their data examining the influence of previous response type on performance. This was to assess the effects of proactive interference on performance as it has been found that a subject's response on the current trial can be influenced by the type of response made on the previous trial (Harper et al., 2005). They revealed that as delay and drug dose increased rats were more likely to be influenced by the previous response type. For example if in the previous trial the rat had responded on the left lever and then in the current trial the correct response was to also respond on the left, then the rat was more likely be correct than if the current trial required them to switch responding by now pressing the lever on the right. Harper et al. (2005) suggested the rats may confuse events between the previous trial and the current trial and it is this confusion that produces the decrease in accuracy seen with acute MDMA exposure in DMTS tasks. Therefore they argued that it may not be working memory or episodic memory per se that is affected by MDMA administration

but that it is reference memory that is impaired where subjects mix up events between trials (Harper et al., 2005). However, many of the tasks used to assess acute effects of MDMA on memory unfortunately do not allow an independent assessment of working memory from reference memory.

The hypothesis that rats become confused about the rules of DMTS tasks was investigated further by Harper, Hunt and Schenk (2006). They examined the acute effects of MDMA in rats and proactive interference was examined by manipulating the inter-trial interval (ITI) during the DMTS task. If rats become confused between the response required on the previous and current trials then increasing the ITI should decrease this deficit as increasing the time interval between trials should reduce the likelihood of them mixing up the previous and current trials (Harper et al., 2006). A larger ITI of 15 seconds produced less disruption in performance than a smaller ITI of 5 seconds supporting their argument that the decrease in accuracy seen with acute MDMA exposure results from a form of proactive interference (Harper et al., 2005).

Therefore DMTS tasks may have a substantial reference memory component and it may be this type of memory that is affected by acute MDMA exposure. However, this is still only speculative as this paradigm does not specifically distinguish between working and reference memory errors.

Test Battery Studies

Other studies have utilised a battery of cognitive tasks to examine the effects of MDMA on cognition. For example Frederick et al. (1995) and Frederick and Paule (1997) examined the acute effects of MDMA administration (0.1 to 1.0 mg/kg) on Rhesus monkeys utilising the Operant Test Battery (OTB). This battery includes different tasks assessing time estimation, short-term memory, attention, motivation,

learning and discrimination. Monkeys were injected intramuscularly with either MDMA or saline thirty minutes before completing the various cognitive tests.

Performance on the time estimation task was severely impaired when they were administered a relatively low acute dose of 1mg/kg of MDMA to the extent that no monkey was able to perform the task. There was also a significant dose dependent decrease in responding on progressive ratio schedules. However acute MDMA administration did not significantly affect accuracy on a conditional discrimination task that assessed the subjects' ability to discriminate between different colours. Learning was assessed using a four lever sequence task where the sequence to be learned changed each testing session. This task assessed subject's ability to change their behaviour and acquire the new sequences. MDMA administration significantly decreased accuracy without affecting response rate. Interestingly subjects produced more acquisition errors (between session errors) than retention errors (within session errors) indicating they had more difficulty learning the new sequences rather than remembering the acquired ones. This type of impairment suggests a difficulty in acquiring task rules that could be interpreted as a reference memory impairment (Kay et al., 2009). Frederick and Paule (1997) argued this finding implied MDMA administration left short-term memory processes unaffected but impaired the acquisition of new information producing a perseverative pattern of responding. In conclusion it appears time estimation, motivation and task acquisition are significantly impaired with acute MDMA administration (Frederick et al., 1995). However performance on other cognitive tests such as a relatively simple discrimination task seemed to be spared (Frederick et al., 1995).

Taffe et al. (2001) also used Rhesus monkeys to study the acute effects of MDMA utilising an array of neuropsychological tests. However, unlike the previous study of Frederick and Paule (1997) they used very large doses of MDMA (2 x 10

mg/kg intramuscularly for 4 days) more similar to those usually administered in chronic studies. Tests included a DNMTS task, a self-ordered spatial search task (SOSS), a reaction time task, a progressive ratio task and finally a bimanual motor task. During the drug treatment week MDMA administration produced significant impairments on performance across all cognitive tasks. A potential limitation of the Taffe et al. (2001) study was that the subjects had been used in a previous study conducted five months earlier that examined the effects of other drugs (scopolamine and amphetamine) which may confound the findings.

Maze Tasks

Radial arm mazes are used to study spatial memory and involve the animal being placed in the middle of the maze and it is allowed to explore the maze (Pearce, 1999). Each arm is baited and therefore the optimal strategy for performing the task it to visit each arm only once. This classic radial arm maze paradigm assesses working memory because the subject has to either remember the arms it has visited or those that remain to be visited (Pearce, 1999). Braida, Pozzi, Cavallini and Sala's (2002) used a classic eight-arm radial maze paradigm to assess the acute effects of MDMA on spatial memory performance. Rats were administered increasing doses of MDMA (1, 2 or 3 mg/kg) and the highest dose impaired working memory. In another experiment a two hour delay was introduced between the fourth and fifth arm choices. This manipulation resulted in a dose dependent deficit in long-term working memory. Acute MDMA administration produced a specific memory deficit without disrupting motor activity or increasing stereotypy (Braida et al., 2002). The arm entry patterns of rats were also disrupted in a dose dependent fashion. Therefore, although Braida et al. (2002) argued

their study produced a working memory deficit, because of the disruption of the arm entry pattern the deficit could be interpreted as a reference memory deficit.

One advantage of the radial arm maze is that it can be manipulated to specifically differentiate between reference and working memory. Olton and Papas (1979) designed a paradigm where a set of arms in the radial maze are always baited with reinforcers and the remaining arms are never baited. A rat is placed in the middle of the maze and is allowed to visit a certain number of arms (the number usually containing reinforcers) in a trial. In order to perform optimally the rat needs to learn to go to arms that contain reinforcers and avoid arms that do not. Also as the arms are not re-baited within a trial the rat must also avoid re-visiting arms within a trial. Therefore, in order to learn this task working memory is required to prevent re-visiting the reinforced arms while reference memory is required to avoid visiting arms that are never baited with reinforcers (Olton & Papas, 1979). In this paradigm a working memory error occurs when a rat re-visits an arm during a trial and a reference memory error occurs when a rat visits an arm that was never reinforced (Olton & Papas, 1979).

This paradigm has been used to examine the effects of several drugs on behaviour in the radial arm maze. For example, Wirsching, Beninger, Jhamandas, Boegman and El-Defrawy (1984) used this paradigm to assess the effects of Scopolamine, an acetylcholine receptor antagonist, on rats using an eight arm radial maze. Wirsching et al. (1984) found the mean number of working memory errors significantly increased during the drug phase. However, the mean number of reference memory errors did not. Therefore, Scopolamine selectively impaired working memory and this effect has been found in other studies examining the effects of Scopolamine (Wang & Tang, 1998 and Pilcher, Sessions & McBride, 1997). Researchers have also found using this paradigm and found the same pattern of more working memory errors

than reference memory errors in studies utilising other drugs (e.g. Levy, Kluge & Elsmore, 1983 with the cholinergic antagonist atropine sulphate).

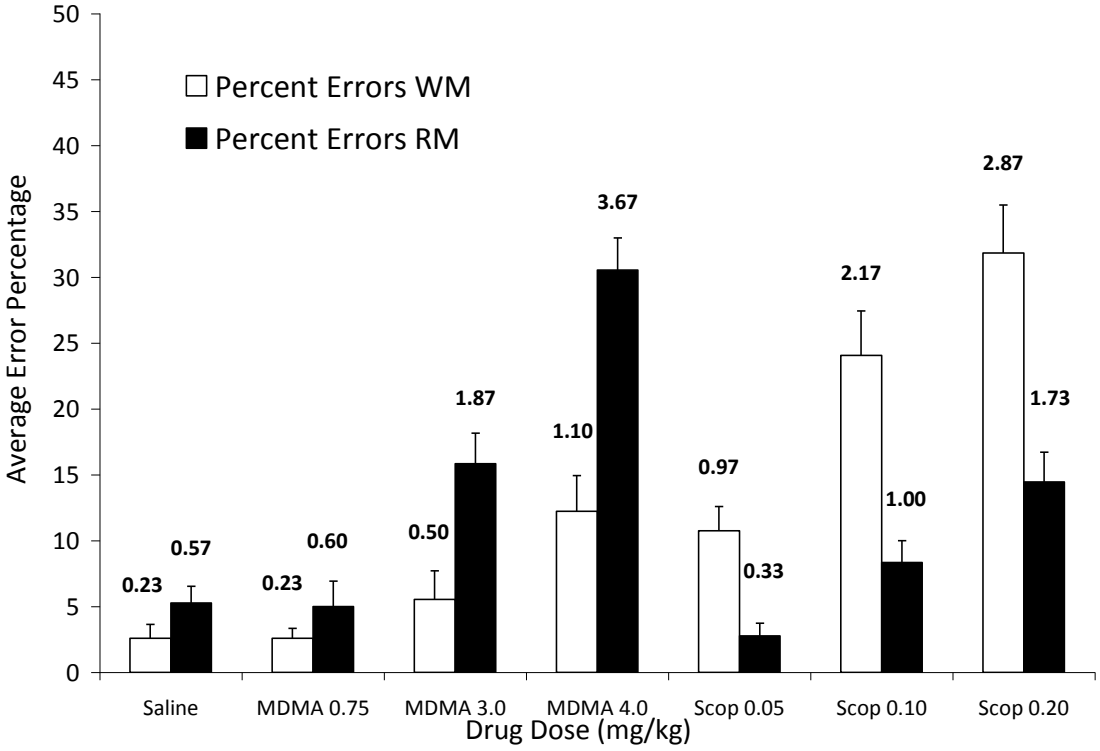

Figure 1: Average error percentage of working memory (WM) and reference memory (RM) errors across all rats for all drug doses replicated from Kay, Harper and Hunt (2009). The values given above each bar are the mean number of total working memory or reference errors made in each condition across rats.

Kay, Harper and Hunt (2009) utilised an eight arm radial to assess the effects of MDMA and Scopolamine on reference and working memory. Fifteen rats were each assigned a set of four arms that were always reinforced and four maze arms that were never reinforced. Each rat was allowed to visit four arms per trial and received three trials per day. As previous studies had found that Scopolamine produced an increase in working memory errors, this drug was used to compare the performance of rats receiving acute doses of MDMA and a saline control. Figure 1 presents findings from Kay et al. (2009) showing the data from working and reference memory errors. It

shows that MDMA produced significantly more reference memory errors than working memory errors while scopolamine produced significantly more working memory errors than reference memory errors.

Conclusion

In summary there seems to be some evidence that acute exposure to MDMA may disrupt reference memory. This is of interest as it seems to be a rather uncommon finding in that many other drugs seem to disrupt working memory instead. This finding therefore warrants further investigation. However, Ecstasy is a complicated drug as it is both a dopamine and serotonin agonist and this makes it difficult to know which neurotransmitter system produces the drug effects seen with MDMA administration (Parrott, 2002).

Many studies have focussed on the effects of MDMA on the serotonin system. In particular there has been a lot of focus on the possible neurotoxic effects of MDMA on serotonin neurons (see General Introduction for a review). However, these studies examine the chronic effects of MDMA exposure that may produce serotonin depletion. Whereas acute exposure produces an increase in serotonin release and the effects of this have not been thoroughly examined. Also dopamine may produce important effects and has largely been under examined in MDMA research (Colado et al., 2004). Therefore, the first section of this thesis aims to partially replicate and extend the work of Kay et al. (2009) by examining which neurotransmitter systems may be responsible for producing the reference memory effect seen with acute exposure to MDMA in the partially baited radial arm maze.

General Acute MDMA Method

Apparatus/Materials

The maze consisted of an aluminium central hub with eight arms radiating from it (see Figure 2). It was secured to an MDF wooden base. The maze arms were 60.5 centimetres (cm) in length, with outer arm walls 9 cm high, inner arm walls 18 cm high and 9.5 cm wide. The centre well of the maze was 30cm in diameter and the maze was situated 81 cm from the ground. At the ends of each arm of the maze there were food wells. These consisted of a small piece of wood, 3 cm high, 2 cm thick and 9 cm wide. A hole 1 cm in diameter and approximately ½ cm deep was drilled out in the top centre of the block to form the well.

Chocolate chips were used as reinforcers and circular plastic Petri dishes that were 5 cm in diameter were attached to the ends of the maze arms to house the chocolate chip reinforcers. Circular velcro dots, 2.3 cm in diameter, were used to attach the dishes to the end of the arms of the maze. During training four open Petri dishes were used without lids to house the obtainable chocolate chips in the reinforced arms. In the non-reinforced arms four other plastic dishes were used that had chocolate chips sealed inside of them. These non-reinforcer dishes were sealed with lids that had several small holes drilled in them. This manipulation allowed the odour of the chocolate chips to permeate from the dishes without allowing the rats to obtain them. This was done to prevent the rats from solving the task using the smell of the chocolate. A digital stopwatch was used to record the amount of time it took a rat to complete a trial. Microsoft Excel for Windows was used to analyse and graph the data. SPSS for Windows, version 11.5, was used to analyse the data.

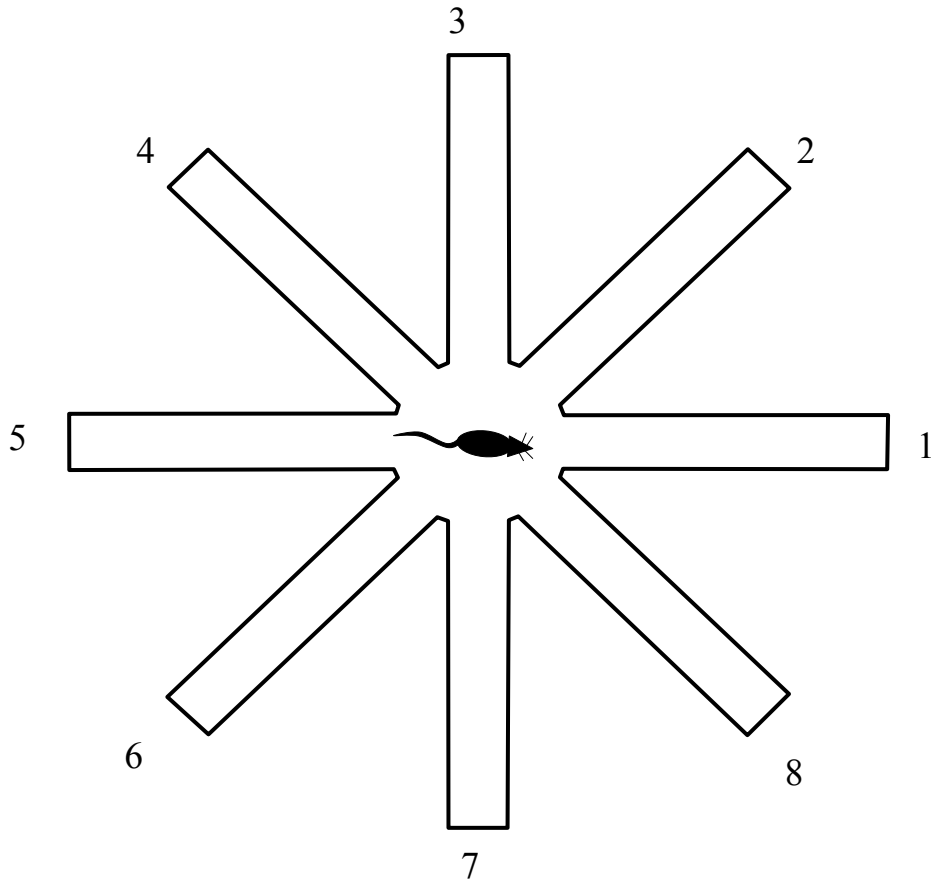

Figure 2: Radial arm maze with rat facing arm number one, the starting position.

Procedure

Pretraining: In an attempt to habituate the rats to the new environment of the maze, they were individually placed inside the centre hub of the maze and were allowed to move around freely. On the first day chocolate chips were placed in the centre of the maze and three chips were placed in each of the arms. One chocolate chip was placed near the opening of the arm, another half way down the arm and one in an open Petri dish at the end of the arm. Rats were given ten minutes (or until all chips were consumed) to explore the maze.

On the second day of habituation rats were again placed in the centre of the maze and allowed to explore for seven and a half minutes (or until all chips were consumed). This time chocolate chips were placed in the middle of the maze and one chip was placed in the centre of each arm while another was placed in the Petri dish at the end of each arm.

Finally on the third day of habituation the rats were placed in the maze for five minutes (or until all the chips were eaten). This time there was one chocolate chip placed in the middle of the maze and one chip in the Petri dish at the end of each arm. On all days of pretraining the arms the rats visited were recorded. This was done to determine if the rats were visiting all arms.

Training: Four reinforcer arms were selected for each rat using a ten-sided die. No more than two consecutive arms were used for each rat and each arm was used approximately the same number of times between rats. Petri dishes without lids were placed in the reinforcer arms and two chocolate chips were placed in the bottom of the dish. Petri dishes with lids, that contained two chocolate chips sealed inside it, were placed in the four remaining non-reinforced arms.

At the beginning of a trial rats were placed in the centre of the maze with their head facing in the direction of arm number one, see Figure 2. The rats were then allowed to enter four arms after which time they were removed from the maze. A choice or arm entry was defined as all four feet passing the line formed between the wood of the centre of the maze and the metal at the beginning of the arm of the maze. These four arm entries constituted a single trial. After a trial was completed and the rat was removed the maze was then re-baited and the rat was placed back in the maze. A set inter-trial interval was not used it was simply the time taken to rebait the maze and

retrieve the rat from the cage which took approximately 15-20 seconds. Each rat received three trials in succession per day.

Arm entries were recorded in the order in which they occurred and error type was also recorded. A working memory error was defined as re-entering an arm already visited during a trial and a reference memory error was defined as entering an arm that had never contained reinforcers. If during a trial a rat re-entered a non-reinforced arm, the first instance was recorded as a reference memory error, while the second visit was recorded as a working memory error. The time it took to complete a trial in seconds was also recorded. Timing commenced from letting the rat go in the centre of the maze, till when all four feet had passed over the entrance of the fourth arm the rat entered. Trial completion time was included as a measure to pick up more sensitive differences in patterns of responding if there were no discernable differences in error type. Chocolate chips in the Petri dishes with lids were replaced daily. The maze was also wiped out each day to remove sawdust and other debris.

Pharmacological Procedure: The acute studies used a within-subjects experimental design with each rat receiving all drug types and doses. The maze running procedure was identical to the training phase during the drug sessions. All drugs (including saline) were administered via an intraperitoneal (i. p.) injection twenty minutes before the rat was placed in the maze.

Statistical Analyses: All inferential statistics were calculated using an alpha level of 0.05. All p-values are given to two decimal places.

Study 1: 5-HT and Dopamine Agonists in the Radial Arm Maze

Serotonin (5-HT)

5-HT is a common chemical found in animals and plants (Sirek & Sirek, 1970) discovered in the late 1940s (Ogren, et al., 2008). Several years later it was detected in the human brain and identified as a neurotransmitter (Ogren et al., 2008). Within the forebrain there are several areas where there are serotonergic axons including the hypothalamus, cortex, hippocampus, amygdala and striatum (Lucki, 1998). 5-HT plays a role in functions such as temperature regulation, pain perception, food consumption, sleep cycles, motor activity, mood, cardiovascular regulation, circadian rhythms, aggression, sexual behaviour and learning (Ruotsalainen et al., 1997; Lucki, 1998).

The study of 5-HT is important in MDMA research because MDMA administration produces changes to the serotonergic system. Acute exposure to MDMA increases 5-HT release (Kish, 2002) while chronic exposure to MDMA has been associated with long-term 5-HT depletion (Marlberg & Bonson, 2001). Studies have examined the effects of MDMA use on serotonergic function using brain imaging techniques such as PET scans have found potentially harmful alterations to the 5-HT system that correlate with Ecstasy use (McCann et al., 1998). Therefore there is evidence suggesting long-term Ecstasy use can lead to alterations in the serotonergic system. However, there has been less research on the acute effects of MDMA on serotonergic function.

5-HT and Cognition

Animal research has found 5-HT plays an important function in various cognitive behaviours with activation of 5-HT receptors producing impairments in learning and working memory in a variety of tasks (Buhot, 1997). A recent review article by Meneses (1999) examined the evidence for 5-HT's importance in cognition and concluded there was support for the hypothesis that 5-HT pathways and receptors are present in brain areas commonly associated with memory and learning. There is also evidence from human research that 5-HT may play an important role in cognition. For instance serotonergic cells in patients with Alzheimer's show damage suggesting 5-HT may play a role in age-related cognitive disorders (Santucci et al., 1996).

Research utilising human participants has found memory impairments correlated with Ecstasy use. These impairments in cognitive function have been correlated with evidence of serotonergic damage where the amount of Ecstasy used tends to be positively related to damage to the serotonergic system and the degree of cognitive impairment (Bolla et al., 1998; McCann et al., 1999). In addition deficits in verbal memory functioning have been found in Ecstasy users and these have also been associated with changes in 5-HT functioning (Reneman et al., 2001). While some studies have found evidence of serotonergic manipulation impairing cognitive performance, some have found it to improve performance while others have found it had no effect (Santucci et al., 1996). Ogren et al. (2008) also point out that confusingly depleting and increasing 5-HT activity have both been shown to impair cognition. Hence the exact nature of 5-HT's role in cognition is still unclear (Meneses, 1999).

It should be noted that studies reporting 5-HT stimulation enhancing cognitive performance often involve participants from clinical populations. For example people suffering from depression (Levkovitz et al., 2002) and schizophrenia (Meltzer &

Sumiyoshi, 2008) have shown improved cognitive functioning after treatment with 5-HT agonists. However in these cases participants may have abnormal brain functioning that may impair cognitive functioning in the first place. Studies reporting improved cognitive functioning after 5-HT stimulation in normal populations are rare. Hence increasing 5-HT activity in healthy participants may not be beneficial to cognition (Barch, 2004). Therefore the focus of this research review, summarised in Table 1, will focus on 5-HT manipulation producing cognitive impairments.

A lot of research has focused on the effects of 5-HT found after chronic MDMA exposure. Several hours after MDMA administration there is a temporary depletion of 5-HT and chronic use of MDMA has been associated with long-term 5-HT depletion (Marlberg & Bonson, 2001) and damage to serotonergic axons (Ricaurte, 1989). Therefore, this research tends to focus on 5-HT depletion and damage to the serotonergic system. Acute administration of MDMA produces an increase in 5-HT activity (Marlberg & Bronson, 2001). This effect has not been studied as extensively as 5-HT depletion but there is evidence that increasing 5-HT release may also produce cognitive impairments (Santucci et al, 1996). For example Santucci et al. (1996) investigated the effects of *p*-chloroamphetamine to rats. Performance on a passive avoidance task and a radial arm maze task were significantly impaired during the 5-HT release phase of the drug but not during the depletion phase suggesting 5-HT release impaired cognitive performance.

Table 1: Summary of research on the effects of serotonin (5-HT) agonists on cognition.

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Cognitive Effects
Lader et al. (1986)	Citalopram – SSRI (20 & 40 mg/kg)	5-HT Agonist	Orally	Healthy Human Volunteers	Battery of cognitive tasks	Significantly impaired immediate recall & coding skills. Did not effect reaction time or delayed memory performance
Winter & Petri (1987)	LSD (0.1 to 1.0 mg/kg)	5-HT Agonist	I.P. injections 15 minutes before testing	Fischer 344 Rats	Standard 8 arm radial maze task (all arms were baited) – assessed spatial working memory	LSD - significant decrease in performance.
	TFMPP (0.3 to 1.0 mg/kg)	5-HT_{2C} Agonist				TFMPP – no significant effect.
	8-OH-DPAT (0.1 t0 3.0 mg/kg)	5-HT_{1A} Agonist				8-OH-DPAT – significantly impaired performance.
	RU 24969 (0.3 to 3.0 mg/kg)	5-HT_{1A} & $_{1B}$ Agonist				RU 24969 – significantly impaired performance.
Rowan et al. (1990)	Buspirone (0.5 to 2.0 mg/kg)	5-HT_{1A} Agonist	I.P. injection 30 mins before testing	Albino Wistar rats	Passive avoidance task (retention tested24 hours later)	Significantly impaired performance on retention test of passive learning.
					Morris Water Maze task – assessed spatial memory	Significantly impaired acquisition (increased time to find platform & impaired probe trials).

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Cognitive Effects
Carli & Samanin (1992)	8-OH-DPAT (30, 100 & 300 µg/kg)	5-HT_{1A} Agonist	S.C. injection 30 mins before testing	Albino Rats	Morris Water Maze – assessed spatial memory.	Accuracy significantly reduced with no effect on latency – spatial memory impaired.
Ohno et al. (1993)	8-OH-DPAT (0.32 & 1.0 mg/kg IP)	5-HT_{1A} Agonist	I.P. injection	Rats	Three panel runway task - assessed spatial memory & differentiated between working and reference memory.	Both intraperitoneal & intra-hippocampal administration of 8-OH-DPAT produced more working memory errors than reference memory errors.
	8-OH-DPAT (1.0, 3.2 & 10.0 µg/side IH)		IH injection			
Jansen & Andrews (1994)	Fluoxetine – SSRI (0.625 to 10 mg/kg)	5-HT Agonist	S.C. injections 30 minutes before testing	Long Evans Rats	Delayed (5 to 45 seconds) matching to position task – assessed spatial memory	Fluoxetine - no significant effect on both tasks
	Fenfluramine – SSRI (0.313 to 5 mg/kg)	5-HT Agonist			Delayed (5 to 45 seconds) nonmatching to position task – assessed spatial memory	Fenfluramine - largest dose significant deficit in performance on both tasks
	Ipsapirone (2.5 to 10 mg/kg)	5-HT_{1A} Agonist				Ipsapirone - no significant effect on accuracy on either tasks but did effect reaction time
Buhot et al. (1995)	8-OH-DPAT (5µg/µl)	5-HT_{1A} Agonist	I.H. injections 15 mins before testing	Long-Evans Black-hooded Rats	8 arm radial maze task (4 arms baited and 4 unbaited)	8-OH-DPAT – no significant effect.
	CP-93,129 (5, 10 & 16 µg/µl)	5-HT_{1B} Agonist				CP-93,129 - significantly more RM errors than WM errors.

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Cognitive Effects
Carli et al. (1995)	8-OH-DPAT (100 µg/kg)	5-HT_{1A} Agonist	S.C. injection 15 mins before testing	Albino Rats	Morris Water Maze task that differentiated between visual & spatial memory	Produced impairment on spatial task but not visual task – stimulation of 5-HT_{1A} receptors impaired spatial learning
Herremans et al. (1995)	Fluvoxamine – SSRI to 10 mg/kg) Ipsapirone (0.3 to 10 mg/kg) TFMPP (0.03 to 0.3 mg/kg) Flesinoxan (0.3 to 3 mg/kg)	5-HT Agonist 5-HT_{1A} Agonist 5-$HT_{1B/1D/2C}$ Agonist 5-HT_{1A} Agonist	I.P. injection 20 mins before testing	Wistar Rats	Delayed (1 to 20 seconds) conditional discrimination task (assessing working memory)	First 3 drugs - significant dose dependent but delay independent impairment in performance (attention/encoding deficit rather than working memory impairment) Flesinoxan - significant delay dependent impairment indicative of a working memory deficit (rate of forgetting)
Robbe & O'Hanlon (1995)	Paroxetine – SSRI (20 & 40 mg/kg)	5-HT Agonist	Orally	Healthy human volunteers	Driving & cognitive performance (including tracking, attention visual discrimination, recognition & memory)	Lower dose – no effect on performance. Larger dose - significant impairments on tracking, divided attention & recognition tasks as well as subjective ratings of memory disturbances.

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Cognitive Effects
Santucci et al. (1996)	p-chloroamphetamine (0.5 to 2.5 mg/kg)	Increases 5-HT release & then 5-HT depletion	I.P. injection 30 mins prior to testing	Sprague-Dawley Rats	Passive avoidance task – assessed learning & long-term retention.	Impaired performance on avoidance task during 5-HT release not depletion phase.
					8 arm radial maze (4 arms baited & 4 arms unbaited).	Significant increase in working & reference memory errors during 5-HT release not depletion phase.
Kant et al. (1996)	8-OH-DPAT (0.25, 0.5 & 1.0 mg/kg)	5-HT_{1A} Agonist	I.P. injection 30 mins before testing	Sprague-Dawley Rats	Modified Morris Water Maze - rats swam through alleyways & doors to find a platform	5-HT_{1A} agonist - significantly more errors & an increase in latency.
	TFMPP (0.25, 0.5 & 1.0 mg/kg)	5-HT_{2C} Agonist				5-HT_{2C} agonist - increase in latency (no effect on accuracy).
Warburton et al. (1997)	8-OH-DPAT (0.05 to 1.0 mg/kg)	5-HT_{1A} Agonist	I.P. injection 30 mins before testing	Listar-Hooded rats	Delayed non-matching-to-position task using operant chambers (delays of 0, 8, 16 & 32 seconds) – assessed spatial working memory	Highest dose – significant delay-independent impairment (increase in premature responding & bias).
	8-OH-DPAT (10, 30, 100 ng)		I.H. 10 mins before testing			Administration into hippocampus - significant delay independent impairment
	8-OH-DPAT (10, 30, 100 ng)		I.R.N. 10 mins before testing			Highest dose into median raphe nucleus - delay independent improvement in performance. Lower doses - no effect.

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Cognitive Effects
Kant et al. (1998)	8-OH-DPAT (0.1, 0.25 & 0.5 mg/kg)	5-HT_{1A} Agonist	I.P. injection 30 mins before testing	Sprague-Dawley Rats	Modified Morris Water Maze as used above. Rats were given 25 trials on a maze configuration before drug administration then maze layout was changed & they assessed how subjects learnt the new maze configuration.	8-OH-DPAT - no effect on learnt maze. New maze - significantly more errors & increased swim times.
	Buspirone (2.5, 5.0 & 10 mg/kg)	5-HT_{1A} Agonist				Buspirone - no effect on learnt maze. New maze - lower doses significantly more errors & increased swim times. Highest dose completely blocked learning of new maze.
	DOI (0.1 & 0.25 mg/kg)	5-HT_2 Agonist				DOI - significantly slower swim times on well learned & new maze. No effect on errors.
Luciana et al. (1998)	Fenfluramine – SSRI (60 mg/kg)	5-HT Agonist	Orally	Healthy human volunteers	Delayed spatial location task (assessed spatial working memory) Spatial location task (assessed motor function) Biletter cancelation task (assessed visual scanning & motor function)	Fenfluramine - significant impairment in spatial memory – especially with longer delays. Other 2 tasks that assessed motor function not significantly affected – therefore fenfluramine produced spatial working memory deficits.

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Cognitive Effects
Naghdi & Majlessi (2000)	Citalopram – SSRI (1, 2, 4, 8 & 16 mg/kg)	5-HT Agonist	I.P. injection 30 mins before testing	Albino Wistar Rats & N.MRI Mice	Morris water maze task – assessed spatial memory Alternating T-maze task, assessed spatial memory	Significantly impaired performance (increase in time taken & distance travelled platform). No effect on T-maze task.
Majlessi & Naghdi (2002)	Citalopram – SSRI (1, 2, 4 & 8 mg/kg) Fluoxetine – SSRI (1, 2, 4, 8, & 16 mg/kg)	5-HT Agonist 5-HT Agonist	I.P. injection 30 mins before testing	Albino Wistar Rats	Morris Water Maze task – assessed spatial memory	Both SSRIs - spatial learning impairment (significant increase in latency & distance travelled to platform without change in swimming speed)
Ahlander-Luttgen et al. (2003)	Anpirtoline (1 to 1.0 mg/kg) NAS-181 (1.0 to 10 mg/kg)	5-HT_{1B} Agonist 5-HT_{1B} Antagonist	S.C. injection 30 mins before testing	Sprague-Dawley Rats	Morris Water Maze – assessed spatial memory Passive avoidance task (retention tested 24 hours later).	Anpirtoline - significant impairment in both tasks. NAS-181 – higher doses significantly altered performance on water maze task. NAS-181 pre-treatment attenuated the impairments produced in both tasks.
Luttgen et al. (2005)	8-OH-DPAT (0.03 to 0.3 mg/kg) NAD-299 (0.05 & 0.5 mg/kg)	5-HT_{1A} Agonist 5-HT_{1A} Antagonist	S.C. injection 15 mins prior to testing	Sprague-Dawley Rats	Morris Water Maze – assessed spatial memory Passive avoidance task (retention tested 24 hours later).	Significantly impaired water maze performance Improved passive avoidance performance at low doses & impaired it at high doses. Pre-treatment with NAD-299 blocked 8-OH-DPAT impairments in both tasks.

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Cognitive Effects
Egashira et al. (2006)	8-OH-DPAT (1 mg/kg)	5-HT_{1A} Agonist	I.P. injections 15 mins before testing	Wistar Rats	Standard 8 arm radial maze task (all arms were baited) – assessed spatial working memory	8-OH-DPAT - significant dose dependent impairment in performance.
	WAY-100635 (0.001 to 0.1 mg/kg)	5-HT_{1A} Antagonist				WAY-100635 - significantly attenuated 8-OH-DPAT impairment.
	NAN-190 (0.3 to 3.0 mg/kg)	5-HT_{1A} Antagonist				NAN-190 - significantly attenuated 8-OH-DPAT impairment.
	8-OH-DPAT (4µg/side)	5-HT_{1A} Agonist	Micro-injections into various brain regions			8-OH-DPAT into dorsal hippocampus significantly impaired performance that was attenuated by administration of NAN-190.
Wadsworth et al. (2005)	Selective serotonin reuptake inhibitors (SSRI)	5-HT Agonist	Orally	Human participants taking SSRIs & drug free controls	Battery of cognitive tasks assessed reaction time, attention & memory.	SSRI treatment - significant effect on episodic memory, recognition memory & delayed recall. No effect on working & semantic memory.

I.P. – intraperitoneal S.C. – subcutaneous I.H – intra-hippocampal infusions I.R.N. - intra-raphe nucleus infusions

To better understand the role of 5-HT in cognitive function other studies have investigated the effects of various 5-HT agonists on cognitive performance. Administration of the 5-HT_{1A} agonist 8-Hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) has significantly spatial learning spatial learning (Carli & Samanin, 1992; Carli, Luschi & Samanin, 1995; Luttgen, Elvander, Madjid & Ogren, 2005; Kant et al., 1996; Kant, Wylie, Chu & Ghosh, 1998). Learning of a passive avoidance task has also shown impairment with higher doses of 8-OH-DPAT (Luttgen et al., 2005). The 5-HT_{1A} agonist Buspirone has also impaired water maze performance (Kant et al., 1998) and impaired passive avoidance learning (Rowan, Cullen and Moulton, 1990). In particular it would appear 8-OH-DPAT impairs working memory in the radial arm maze (Winter & Petti, 1987; Egashira et al., 2006), DNMP performance (Warburton, Harrison, Robbins & Everitt, 1997) and in delayed conditional discrimination performance (Herremans et al., 1995).

The role of 5-HT_{1B} receptors in cognition has also been examined where administration of various 5-HT_{1B} receptor agonists has been found to impair performance on a passive avoidance task, a water maze task (Ahlander-Luttgen, Madjid, Schott, Sandin & Ogren, 2003) and a radial arm maze task (Winter & Petti, 1987). However not all 5-HT receptors appear to play an important role in cognition. For example 5-HT_{2C} agonists have not impaired performance on a radial arm maze task (Winter & Petti, 1987) and have not affected accuracy in water maze tasks (Kant et al., 1996; Kant, Wylie, Chu & Ghosh, 1998).

Ohno, Yamamoto and Watanabe (1993) utilised a three panel runway task and found 8-OH-DPAT produced more working memory errors than reference memory errors. Ohno et al. (1993) concluded 8-OH-DPAT impairs spatial memory, specifically working memory. With relevance to the current thesis there are also studies that have used the partially baited radial arm maze to examine the effects of 5-HT stimulation on

reference and working memory errors in rats. Buhot, Patra and Naili (1995) trained rats at this task prior to administration of intra-hippocampal injections of 8-OH-DPAT and CP-93,129. However unlike previous research they found 8-OH-DPAT had no effect on performance. Also contrary to previous research Buhot et al. found CP-93,129 produced significantly more reference memory errors rather than working memory errors. Therefore, the serotonergic effects on working and reference memory are not clear.

The research summarised above clearly supports the view that various 5-HT receptors may be involved in learning and memory. However more research is needed to clarify the different effects of various 5-HT agonists on cognition, especially working and reference memory processes. However on balance there does seem more evidence that stimulating 5-HT_{1A} receptors, impairs working memory more than reference memory processes. Therefore while acute MDMA exposure increases 5-HT release it would appear unlikely that this increase in 5-HT activity produced the reference memory impairments seen with acute MDMA exposure in Kay et al. (2009).

Selective Serotonin Reuptake Inhibitors (SSRIs)

Rather than examining the role of the individual 5-HT receptors on learning and memory other researchers have focussed on more general 5-HT agonists. Common forms of 5-HT agonists are SSRIs which are indirect agonists as they alter the serotonergic system by selectively inhibiting 5-HT reuptake into the pre-synaptic neuron (Majlessi & Naghdi, 2002). This increases 5-HT by leaving more 5-HT in the synapse and therefore increasing extracellular concentrations of 5-HT and serotonergic transmission (Naghdi & Majlessi, 2000). This drug action is relevant as MDMA also inhibits 5-HT reuptake (Marlberg & Bonson, 2001) and there have been accounts of

cognitive impairments in relation with taking SSRIs (Wadsworth, Moss, Simpson & Smith, 2005).

Administering SSRIs to human participants has resulted in impaired performance on immediate recall memory and coding tasks (Lader, Melhuish, Grcka, Overo & Christensen, 1986) as well as impairments in attention and recognition tasks (Robbe & O'Hanlon, 1995). Also subjectively participants have reported side effects including memory disturbances (Robbe & O'Hanlon, 1995). Luciana, Collins and Depue (1998) found Fenfluarmine (quickly releases 5-HT from pre-synaptic terminals & inhibits reuptake) impaired spatial working memory.

Utilising animal subjects Jansen and Andrews (1994) examined the effects of 5-HT activity on spatial memory in rats using a DMTP and a DNMTP task. Once performance had stabilised they administered various 5-HT agonists. The SSRI Fluoxetine failed to significantly impair performance in either task whereas the 5-HT release enhancer and SSRI Fenfluramine produced a significant impairment on both tasks. Jansen and Andrews (1994) argued these findings suggest more of a general disruption in behaviour rather than a specific effect on cognition and further highlight the difficulty in identifying the effects that 5-HT has on cognition as drugs that have very similar effects on 5-HT produced different effects (Jansen & Andrews, 1994).

The SSRI Fluvoxamine has produced delay independent impairments on a delayed conditional discrimination task suggesting it impaired attention or encoding (Herremans et al., 1995). More recently Naghdi and Majlessi (2000) found the SSRI Citalopram did not significantly affect performance on a T-maze task but did produce a significant dose-dependent deficit on performance in the Morris water maze. In a similar study Majlessi and Naghdi (2002) examined the acute effects of Citalopram and Fluoxetine on acquisition of the Morris water maze. Both SSRIs produced

significant dose dependent impairments on performance. However, neither drugs produced a change in the swimming speed of the rats indicating motor activity was not impaired. Therefore, Majlessi and Naghdi (2002) argued the learning deficit was due to an impairment in spatial learning produced by the 5-HT agonists.

In conclusion there does seem to be strong evidence that an increase in 5-HT levels, either by stimulating specific 5-HT receptors or increasing 5-HT levels by inhibiting reuptake is associated with deficits or impairments in cognitive function. While not conclusive the majority of studies seems to implicate a role of 5-HT in working memory. However, very few studies have specifically tried to differentiate the acute effects of drugs that act as 5-HT agonists on working and reference memory. Clearly further research in this area of study would be valuable considering the number of people who take SSRIs and the number of people who are taking Ecstasy.

Dopamine

Dopamine was first discovered in the central nervous system in the late 1950s (Beninger, 1983). It is a neurotransmitter that plays an important role in many functions including locomotor activity, emotion, cognition and neuroendocrine secretion (Jaber et al., 1996). Dopamine has also been shown to play a role in reinforcement or motivation (Nieoullon, 2002) as well as being associated with various forms of learning (Beninger, 1983). Disruptions of dopaminergic function have been associated with several diseases and conditions such as Parkinson's disease (PD), schizophrenia, Tourette's syndrome, attention deficit hyperactive disorder (ADHD), pituitary tumours (Vallone et al., 2000), Alzheimer's disease, Huntingdon's chorea, autism and bipolar disorders (Nieoullon, 2002). In addition disruptions to

dopaminergic function have been associated with drugs of abuse such as cocaine and amphetamine that increase dopamine activity in the brain (Beninger, 1983).

The reason dopamine is important in regards to MDMA research is that MDMA exposure produces a rapid increase in dopamine release (Colado et al., 2004). In some areas of the brain MDMA exposure produces a larger increase in extracellular dopamine than that of 5-HT (Colado et al., 2004). MDMA increases dopamine release in two ways. Firstly by reversing the dopamine uptake carrier and secondly by stimulating the 5-HT_{2A} receptors (Liechti & Vollenweider, 2001). In animal research a number of studies have found repeated exposure to MDMA produces changes in the dopaminergic system in experimental animals with acute administration of the drug producing an increase in dopamine release (Gerra et al., 2002).

However unlike the substantial amount of research that has produced evidence that MDMA's action on the serotonergic system produces neurotoxic brain damage, there is little indication that MDMA produces any permanent impairment to the dopaminergic system (Colado et al., 2004). In fact there is very little evidence that long-term use of MDMA produces damage to dopamine neurons in humans or rats (Colado et al., 2004).

Dopamine and Cognition

The dopaminergic system may be vital in short-term or working memory (Gouzoulis-Mayfrank et al., 2003) and has also been associated with some forms of learning (Beninger, 1983). Evidence from studies support the notion that dopamine functioning plays an important role in a range of cognitive processes (Cropley, Fujita, Innis & Nathan, 2006) including working memory (Watanabe, Kodama & Hikosaka, 1997). In addition a variety of experimental studies using animals have suggested

repeated exposure to MDMA induces dysfunction in the dopaminergic system that may affect learning and memory (Gerra et al., 2002). In rats MDMA administration has produced changes in dopamine neurotransmission in the nucleus accumbens(Gerra et al., 2002), the hippocampus (Shankaran & Gudelsky, 1998; Gerra et al., 2002) and the striatum (Shankaran & Gudelsky, 1998) which are areas that are in important in memory and learning (Meneses, 1999; Jay & Dunnett, 2007).

There is also evidence from imaging and lesioning studies that the striatum plays an important role in procedural learning and the learning of new skills (Jay & Dunnett, 2007) that involve reference memory processes. As the striatum has reliably been found to release dopamine following MDMA administration (Shankaran & Gudelsky, 1998) the MDMA induced reference memory impairments seen in Kay et al. (2009) may be the result of changes to dopamine activity.

A more common method of examining the role of the dopaminergic system on cognition and memory has been to administer dopamine agonists to examine the effect they have on cognitive tasks. Commonly used dopamine agonists are drugs that treat various psychological disorders associated with dopaminergic function. While some studies have found that manipulating dopamine impairs performance, some have found that low doses of dopamine may improve cognitive performance (Barch, 2004). However many of the studies have used abnormal clinical populations that may have abnormal brain functioning in the first place which makes it difficult to ascertain whether dopamine stimulation would actually improve memory function in normal subjects.

In fact the relationship between dopamine function and cognition is complicated and has been described as having an inverted U shaped function where either an increase or decrease in dopamine activity seems to disrupt cognitive performance

(Floresco & Magyar, 2006). Therefore administering dopamine agonists to healthy subjects would be less likely to improve memory function than administering them to subjects that have decreased dopamine functioning (Barch, 2004). Many dopamine agonists are stimulants and hence in low doses they have been shown to improve reaction time (Barch & Carter, 2005). However this does not necessarily mean they alter memory processes per se. Therefore the focus of this research will be on studies that have found evidence of dopamine manipulation producing cognitive impairment and this research is summarised in Table 2.

As seen in Table 2 Shohamy et al.'s (2006) work examining learning in PD patients showed patients on L-dopa were impaired at acquiring an associative learning task. Shohamy et al. (2006) concluded dopamine functioning is involved in reward and feedback based learning. In addition this form of impairment suggests an impairment in the ability to learn task rules which may indicate a reference memory deficit. Further evidence that dopamine may play a role in reference memory processes also comes from PD patients. Procedural memory is a term used in human cognition research that relates to the gradual acquisition of fixed rules and procedures of cognition, perception or motor activity (Thomas-Ollivier, Reymann, Moal, Schuck, Lieury & Allain, 1999). This definition of procedural memory has some common features with reference memory learning used in animal research. Procedural memory is assessed by having participants perform two tasks simultaneously. After an initial learning phase on one task a secondary one is introduced. If the first task has been automated (learnt to a procedural level) then there should be minimal interference when another task is introduced (Thomas, Reymann, Lieury & Allain, 1996). Studies using PD patients have shown evidence of impaired procedural memory (Thomas et al., 1996; Thomas-Ollivier et al., 1999). PD patients who showed impaired procedural memory also performed badly on the Wisconsin Card Sorting Task (Thomas-Ollivier et al., 1999).

This suggests they were impaired in their ability to acquire task rules which is also indicative of a reference memory type impairment.

However, one difficulty in examining PD patients is that they have abnormal brain functioning as their condition reduces dopamine levels and the dopamine stimulating medication is administered in an attempt to return their levels to normal. In the current study healthy rats, presumably with normal dopamine functioning, were administered dopaminergic stimulating drugs to increase their dopamine levels beyond normal. Therefore, it may be problematic in extending these findings to the current paradigm. However these findings may reveal that there is an ideal level of dopamine activity required for effective procedural or reference memory performance.

There have been researchers that have administered dopamine agonists to healthy human volunteers. Dopamine agonists have been found to significantly impair the acquisition of an associate learning, learning transference and long-term retention (Breitenstein et al., 2006). They have also significantly impaired performance on tasks that assessed source recognition, item recognition and proactive memory interference (Montoya et al., 2008). Therefore dopamine manipulation produced deficits in learning task rules and confusion or interference on cognitive tasks which may indicate reference memory deficits.

Animal research also provides evidence that administering dopamine agonists can impair cognition such as deficits in long-term memory (Zarrindast et al., 1992), memory formation (Chuhan & Taukulis, 2006), DMTS performance (Kesner et al., 1981; Branch & Dearing, 1982; Baron & Wenger, 2001; Wright & White, 2003; Harper et al., 2005), DNTMS performance (Kesner et al., 1981) time estimation, progressive ratio performance, conditioned position and sequence learning (Mayorga et al., 2000).

Table 2: Summary of research on the effects of dopamine agonists on cognition.

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Memory Effects
Kesner et al. (1981)	d-amphetamine (0.33, 1.0, 2.0 & 3.0 mg/kg)	DA Agonist	I.P. injection 30 mins before testing	Long-Evans rats	DNMTS task using an operant chamber (delays of 0, 5, 15, 30 & 40 seconds)	Larger doses - significant decreases in accuracy & as dose increased the impairment was evident at earlier delays.
					Spatial delayed matching-to-sample task using an 8 arm radial maze (delays of 1 minute or 30 minutes)	2 highest doses - significant increase in errors with the 1 minute delay. But with the 30 minute delay only 3mg/kg dose significantly reduced accuracy.
Branch & Dearing (1982)	Cocaine (0.56 to 10 mg/kg)	DA Agonist	I.M. injection	Pigeons	DMTS task using operant chambers (delays of 0.05 - 4.0 seconds)	Cocaine - dose related significant decrease in accuracy.
Buresova & Bures (1982)	Amphetamine (1 mg/kg)	DA Agonist	I.P. injections 10 mins before testing	Hooded rats	Radial arm maze (12 and 24 arm versions - standard paradigm where all arms were baited.)	Neither drug significantly impaired performance on the standard 12 or 24 arm maze performance.
	Apomorphine (0.05 mg/kg)	DA Agonist			Also involved a condition where a 5 minute delay was introduced between the 6^{th} & 7^{th} arm choice for the 12 arm maze.	Amphetamine (but not apomorphine) produced a significant impairment when the delay was introduced into the maze paradigm.

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Memory Effects
Beatty et al. (1984)	Amphetamine (0.5, 1.0 & 2.0 mg/kg)	DA Agonist	I.P. injection	Albino rats	12 arm radial maze task (6 arms baited & 6 arms not baited). 12 arm radial maze task (6 arms baited & 6 arms not baited). Rat removed for a delay (0 or 5 minutes) after they had visited 3 arms & then put back in maze.	Amphetamine – no increase in either error type compared to saline controls. Significant increase in both WM & RM errors with the 2.0 mg/kg dose of amphetamine but only with the 5 minute delay – DA agonist only produced memory deficit when a delay was introduced.
Zarrindast et al. (1992)	Apomorphine (0.06 to 0.5 mg/kg)	DA Agonist	S.C. injection 30 mins before testing	Albino mice	Active avoidance learning task using mild foot shock. Tested 24 hours later to measure retention (assessed long-term retrieval)	Apomorphine – low doses improved performance. Higher doses - significant impairment in performance (over-stimulating dopamine impairs long-term retention).
Thomas et al. (1996)	PD patients – abnormal dopamine function			Parkinson's disease patients and elderly and student controls	Tactile maze task: First phase was acquiring the maze task. Second phase they had to memorise visual items while concurrently performing the maze task. Arithmetic/alphabet task: First phase involved having to learn a numerical and alphabetical code and solving problems using the code. Second phase completing longer & harder problems.	PD patients - significant impairment in acquiring the maze task compared to controls. During second phase PD patients also showed significant impairment suggesting procedural memory impairment. PD patients - significantly impaired compared to student controls but not age matched controls. In second phase PD patients failed to automate the initial task into long-term memory suggesting a procedural memory deficit.

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Memory Effects
Thomas-Ollivier et al. (1999)	PD patients – abnormal dopamine function			Parkinson's disease patients and age matched controls	Verbal material task: First phase involved memorising a poem. Second phase introduced a secondary concurrent finger tapping task	PD patients - significantly more errors than controls when learning the poem. During second phase of the task PD patients were significantly impaired compared to controls - had more difficulty in performing two tasks at once (impaired procedural memory).
					Visuo-motor task: First phase involved learning & memorising sequence of key presses on a keyboard Second phase introduced a secondary concurrent task that required them to keep track of alphabetical stimuli	PD patients - significant impairment in acquiring the visuo-motor task compared to controls. During second phase PD patients did not score differently from controls suggesting they were able to perform these two tasks concurrently as well as controls.

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Memory Effects
Mayorga et al. (2000)	Methylphenidate (MPH) (1.12 to 18 mg/kg) Amphetamine (0.1 to 6 mg/kg)	DA Agonist	I.P. injection 15 mins before testing	Sprague-Dawley rats	Battery of operant tasks consisting of: A conditional position task (having to learn to discriminate between tones and lights)	Lower doses - both drugs produced an increase in response rate. Higher doses - both decreased response rate. Both MPH & amphetamine at higher doses produced a significant decrease in accuracy.
					An incremental repeated acquisition task (learning lever sequences)	Both drugs increased response rates at lower doses but at higher doses decreased response rates. Both drugs significantly decreased accuracy at higher doses.
					Temporal response task (assesses sensitivity to the passage of time)	Significantly impaired performance at lower doses than the other cognitive tasks – performance easily disrupted.
					Motivation task (using a progressive ratio schedule)	At higher doses rats were less inclined to work for reinforcement - however effect only significant for amphetamine.
Baron & Wenger (2001)	d-amphetamine (0.01 to 1.0 mg/kg) Cocaine (0.1 to 3.2 mg/kg)	DA Agonist DA Agonist	I.M. injections into the upper leg	Squirrel monkeys	DMTS task using operant chambers with a fixed delay for three seconds.	Both drugs produced a significant reduction in accuracy compared to saline.

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Memory Effects
Wright & White (2003)	Methylphenidate (MPH/Ritalin) (0.25, 2.5 & 10 mg/kg)	DA Agonist	I.P. injection 30 mins before testing	Pigeons	DMTS task using operant chambers (delays of 0.2, 1, 3, 6 & 12 seconds).	Highest dose significantly reduced accuracy in both the FR1 & FR5 conditions indicating manipulating attention did not alter performance.
					In sample phase used FR1 & FR5 schedules to manipulate attention/encoding	MPH affected accuracy in a delay-independent fashion – suggesting encoding deficit rather than a memory deficit per se.
Harper et al. (2005)	Cocaine (0.3, 1.0, 2.0 & 3.0 mg/kg)	DA Agonist	I.P. injections ten mins before testing	Sprague-Dawley rats	DMTS task using operant chambers with delays of 0.1, 3.0, 9.0 & 18.0 seconds.	Both drugs - dose dependent impairment in performance (significant decrease in accuracy) but in a delay independent manner - attention or encoding deficit.
	Amphetamine (0.1, 0.3, 0.6 & 1.0 mg/kg	DA Agonist			Analysed data for evidence of proactive interference	Both drugs - proactive interference effect where rats were more likely to be influenced by the response on the previous trial (confusion between trials/task rules implying reference memory deficit)
Breitenstein et al. (2006)	Pergolide (0.1 mg)	DA Agonist	Orally 2 hours before testing	Healthy human volunteers	Acquisition of associative learning task	Significant acquisition impairment.
					Transfer of learning task	Significantly impaired transfer of learning.
					Long-term retention assessed a week & a month later	Significant impairment on both retention tests.

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Memory Effects
Chuhan & Taukulis (2006)	Methylphenidate (MPH) (10 mg/kg)	DA Agonist	Orally	Long-Evans rats	Object recognition task with 24 hour delay (non-spatial episodic memory)	Significantly less time exploring novel object – impaired memory formation.
Shohmay et al. (2006)	L-dopa (normal medical dosage)	DA Agonist	Orally	PD patients taking L-dopa & PD patients who ceased medication & PD free controls	Acquisition of an associative learning task	L-dopa patients - significant impairment on task acquisition.
					Transfer phase - generalise set of response rules	No effect on transfer learning.
					Error-correcting feedback learning task (shaping condition)	L-dopa patients - did not differ significantly from controls with shaping condition.

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Memory Effects
Montoya et al. (2008)	Apomorphine (5 μg/kg)	DA Agonist	S.C. injections 10 mins before testing	Healthy human volunteers	Battery of cognitive tasks: Source & item recognition test Memory interference test (assessed proactive interference) Categorised words tests (immediate recall task)	Apomorphine administration significantly reduced accuracy (compared to controls) on the source & item recognition test. Suggesting participants given the dopamine agonist were impaired at a recognition task & also impaired about where they had seen the stimuli. Apomorphine administration also significantly reduced accuracy on the memory interference test. Suggesting dopamine manipulation produced impairment or confusion on a task measuring interference.
					Go/no-Go test (measured inhibitory control) Stroop test (measures attention & inhibitory processes) Trail making test (assessed visuomotor coordination & executive function) Verbal fluency test (generating words beginning with a particular letter or semantic category)	Accuracy was not significantly reduced on the remaining tasks.

I.M. – intra-muscular I.P. – intraperitoneal S.C. - subcutaneous

The dopamine agonist amphetamine has also impaired working memory in a standard radial maze task but only when a 5 minute delay was introduced into the task (Buresova & Bures, 1982). To specifically examine working and reference memory processes, Beatty, Bierley and Boyd (1984) utilised the partially baited radial arm maze to assess the performance of rats administered amphetamine. Amphetamine administration produced a significant increase in both working and reference memory errors but only when the paradigm was altered by introducing a delay in between arm visits. It thus appears that amphetamine can disrupt memory processes providing there is a delay between the to-be-remembered event and testing (Beatty et al., 1984).

Harper et al. (2005) examined performance on a DMTS task for evidence of proactive interference and found for all drugs (amphetamine, cocaine & MDMA) the same pattern emerged: as drug dose increased rats were more likely to be influenced by the previous response type. Therefore, although previous researchers have interpreted this impairment as affecting attention or encoding Harper et al. (2005) argued rats did not have impaired attention because this would imply that rats were not attending to previous trial events. Instead Harper et al. (2005) argued the rats may confuse events between the previous trial and the current trial. This confusion is what produced the decrease in accuracy seen with acute MDMA, cocaine and amphetamine exposure in DMTS tasks. Thus MDMA and the dopamine agonists actually impair reference memory processes as subjects mix up events between trials and become confused as to the rules of the DMTS task (Harper et al., 2005).

In conclusion the finding that drugs and psychological conditions that increase dopamine activity as well as those that block or decrease dopamine activity both have produced deficits in cognitive performance. This has led researchers to argue that there is an optimal level of dopamine required to adequately perform various cognitive tasks (Wright & White, 2003). There is substantial evidence that administration of various

dopamine agonists disrupt cognitive performance on a number of tasks. However, the exact nature of the cognitive impairment produced by drugs that stimulate or mimic dopaminergic activity are still unclear. There is some evidence that dopamine plays a role in working memory with stimulation of the dopaminergic system disrupting short-term memory. However, there is also evidence that stimulating the dopaminergic system may disrupt procedural and reference memory process as well.

The Current Investigation

While there is ample evidence that MDMA administration produces memory impairments, to date no one has been able to say whether the memory deficits seen when MDMA is administered are due to 5-HT or dopamine release. Identifying the role of these two neurotransmitters is important when considering ways of treating or ameliorating the drugs effect on cognitive processes. For example it has been suggested that administering 5-HT_2 antagonists may prevent damage produced by large doses of MDMA (Marlberg & Bonson, 2001) but the validity of this depends on the extent to which cognitive impairments relate to 5-HT as opposed to dopamine. To further examine the effects that neurotransmitters and receptors have on cognition may also be helpful in developing pharmacological treatments for learning and memory impairments (Meneses, 1999).

The current study examined whether the reference memory deficit Kay et al. (2009) observed in the radial arm maze was due to dopaminergic or serotonergic activity within the brain. The review of previous research suggests that 5-HT may play a stronger role in working memory processes than reference memory processes. The current study utilised the 5-HT and dopamine agonists, Citalopram and GBR12909 to examine what effect these drugs have on performance in the radial arm maze.

Citalopram is a potent serotonin agonist that works by selectively inhibiting 5-HT reuptake thus increasing levels of extracellular serotonin (Majlessi & Naghdi, 2002). It is a selective inhibitor of 5-HT and has been described as a useful instrument in examining the function of the serotonergic system (Naghdi & Majlessi, 2000).

GBR12909 is an indirect dopamine receptor agonist (Ellinwood, Davidson,Yu, King & Lee, 2002) as it is a dopamine reuptake inhibitor (Baumann, Charr, Goodman, Ayestas & Rothman, 1995) which is long lasting (Elmer et al., 1996) and has been described as an excellent pharmacological means by which to study the specific role of dopamine in psychostimulant effects and reinforcement (Roberts, 1993).

Based on the literature review it was hypothesised that the acute administration of the 5-HT agonist Citalopram would produce a significant increase in working memory errors compared to reference memory errors in the radial arm maze. It was also hypothesised that due to the role that dopamine function appears to play in procedural and reference memory that the acute administration of the dopamine agonist GBR12909 would produce significantly more reference memory errors than working memory errors.

Method

Subjects

The subjects were fourteen white male Sprague-Dawley rats that were twelve to thirteen months old at the beginning of the study. These rats had been used previously in an Honours research project where they had experienced the training outlined in the General Acute MDMA Method. The rats were kept at approximately 85-90% (between 233 and 281 grams) of their free feeding body weight and began re-training around a

week after reaching this weight. They had continuous access to water and were kept on a 12:12-hour light:dark cycle and were run during the dark phase of this cycle.

Apparatus/Materials

The experiment was carried out in the aluminium maze previously described in the general method section and chocolate chips were used as reinforcers. A digital stopwatch was used to record the amount of time it took a rat to complete a trial.

Drugs used were GBR 12909 (10, 20 & 30 mg/kg), Citalopram (15 & 30 mg/kg), saline (0.9 %) and MDMA (4.0 mg/kg). The Citalopram and MDMA were dissolved in saline to the required dose in 0.9 % of saline solution. The GBR 12909 was also dissolved in the same saline solution. However this drug was more difficult to get into solution and was therefore given the additional treatment of gentle heating and agitation.

Procedure

Subjects completed twelve retraining sessions to bring their performance on the task to the criterion of 80% accuracy. The study was a within-subjects experimental design with each rat receiving all drug types and doses. All drugs were administered via an intraperitoneal (i.p.) injection twenty minutes before running.

Rats were run in groups where the first four rats were injected and then twenty minutes after the first rat was injected all four rats were run in the experiment. Once this group had completed running the maze the second group was run and then the third group of three rats was run and then the final group of three rats were run. There were at least three days between drug sessions.

Table 3: Counterbalancing schedule of drug administration

	Drug Session								
Rat	1	2	3	4	5	6	7	8	9
C1	Saline	Low GBR	Hi GBR	Saline	Low Cital	Hi Cital	Saline	MDMA	GBR 30 mg/kg
C3	Saline	Low GBR	Hi GBR	Saline	Low Cital	Hi Cital	Saline	MDMA	GBR 30 mg/kg
C4	Saline	Low GBR	Hi GBR	Saline	Low Cital	Hi Cital	Saline	MDMA	GBR 30 mg/kg
C6	Saline	Low GBR	Hi GBR	Saline	Low Cital	Hi Cital	Saline	MDMA	GBR 30 mg/kg
C7	Saline	Low GBR	Hi GBR	Saline	Low Cital	Hi Cital	Saline	MDMA	GBR 30 mg/kg
C8	Saline	Low GBR	Hi GBR	Saline	Low Cital	Hi Cital	Saline	MDMA	GBR 30 mg/kg
C9	Saline	Low GBR	Hi GBR	Saline	Low Cital	Hi Cital	Saline	MDMA	GBR 30 mg/kg
C10	Saline	Hi Cital	Low Cital	Saline	Hi GBR	Low GBR	Saline	MDMA	GBR 30 mg/kg
C11	Saline	Hi Cital	Low Cital	Saline	Hi GBR	Low GBR	Saline	MDMA	GBR 30 mg/kg
C12	Saline	Hi Cital	Low Cital	Saline	Hi GBR	Low GBR	Saline	MDMA	GBR 30 mg/kg
C13	Saline	Hi Cital	Low Cital	Saline	Hi GBR	Low GBR	Saline	MDMA	GBR 30 mg/kg
C14	Saline	Low Cital	Hi Cital	Saline	Hi GBR	Low GBR	Saline	MDMA	GBR 30 mg/kg
C15	Saline	Low Cital	Hi Cital	Saline	Hi GBR	Low GBR	Saline	MDMA	GBR 30 mg/kg
C16	Saline	Low Cital	Hi Cital	Saline	Hi GBR	Low GBR	Saline	MDMA	GBR 30 mg/kg

* Low GBR - GBR12909 10 mg/kg
* Hi GBR - GBR12909 20 mg/kg
* Low Cital - Citalopram 15 mg/kg
* Hi Cital - Citalopram 30 mg/kg
* Saline - 0.9 mg/kg
* MDMA - MDMA 4.0 mg/kg

The maze running procedure was identical to the training phase (outlined in the General Acute MDMA Method section) during the drug sessions. Saline 0.9 % was used to obtain a baseline measure to compare the other drug doses with. The rats also received a dose of 4.0 mg/kg of MDMA to examine if Kay et al.'s (2009) findings would be replicated and to compare session performance with the dopamine and serotonin agonists. This dose was chosen as it produced the largest effect in Kay et al.'s (2009) study.

A complete counterbalance of drug type and dose was not feasible because of the difficulties in mixing GBR12909 such that only one dose could be used per testing

day. Thus rats were exposed to the drug type and dosage conditions according to a modified counterbalancing scheme as shown in Table 1. Following these conditions a larger dose of GBR12909 (30 mg/kg) was administered. This condition was included as data from the lower doses indicated a pattern of errors consistent with a pattern of errors resulting from reference memory impairment albeit less severe, seen with administration of MDMA.

Results

In all figures error bars show standard error of the mean. Data from the three test days for each condition were combined. Percent correct figures were calculated by averaging across the three daily trials to obtain an average level of performance for the session for each rat. The data for the three saline sessions were averaged together as visual inspection of the data showed no obvious differences or trends in the data. These data are presented in Figure 3 and show that percent correct values decreased in a dose dependent fashion for both GBR12909 and Citalopram. However, this figure clearly shows that neither of these drugs produced the degree of impairment that MDMA 4.0 mg/kg produced.

A one-way repeated measures ANOVA comparing percent correct for saline and GBR12909 was conducted. There was a significant effect for dose, $F(3, 39) = 6.69$, $p < 0.05$ ($p = 0.00$). A one-way repeated measures ANOVA was also conducted to compare percent correct for saline with Citalopram and a significant effect for dose was found, $F(2, 26) = 5.40$, $p < 0.05$ ($p = 0.01$). Finally a repeated measures t-test revealed a significant effect between saline and MDMA, $t(13) = 7.78$, $p < 0.05$ ($p =$

0.00). Therefore, our findings showed that as drug dose increased for all drugs accuracy significantly decreased.

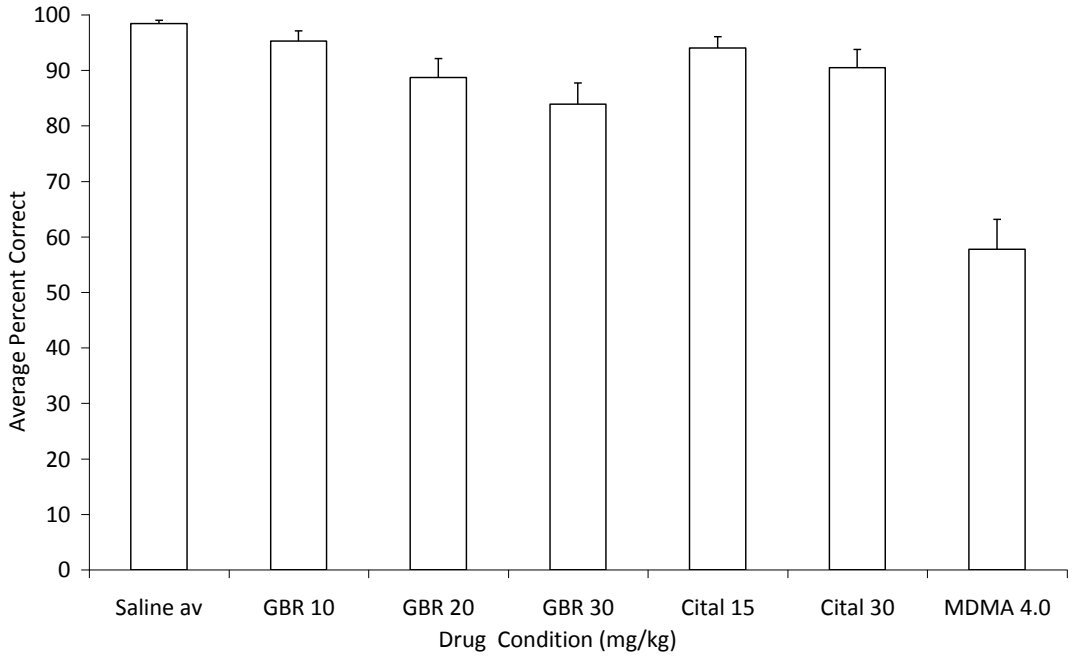

Figure 3: Average percent correct across all rats for each drug and dose.

Mean daily/session trial completion times, in seconds, were calculated for each rat by averaging the three trial completion times from each trial for each drug dose. Again, the three saline doses were averaged together. Figure 4 indicates that generally trial completion times increased as drug dose increased for all drug types, with the exception that the highest dose of GBR12909 (30 mg/kg) produced a faster trial completion time than the immediately smaller dose. As indicated from the error bars some rats were more affected by some doses of the drugs than others.

A one-way repeated measures ANOVA comparing trial completion times for saline and Citalopram revealed a significant effect, $F(2, 26) = 5.94$, $p < 0.05$ ($p =$

0.01). A one-way repeated measures ANOVA was also calculated to compare the trial completion times for saline with GBR12909 and it also produced a significant effect, $F(3, 39) = 3.67$, $p < 0.05$ ($p = 0.02$). Finally a paired samples t-test showed that there was a significant effect for trial completion time between saline and MDMA, $t(13) = -5.68$, $p < 0.05$ ($p = 0.00$). Therefore, as drug dose increased trial completion time was significantly affected.

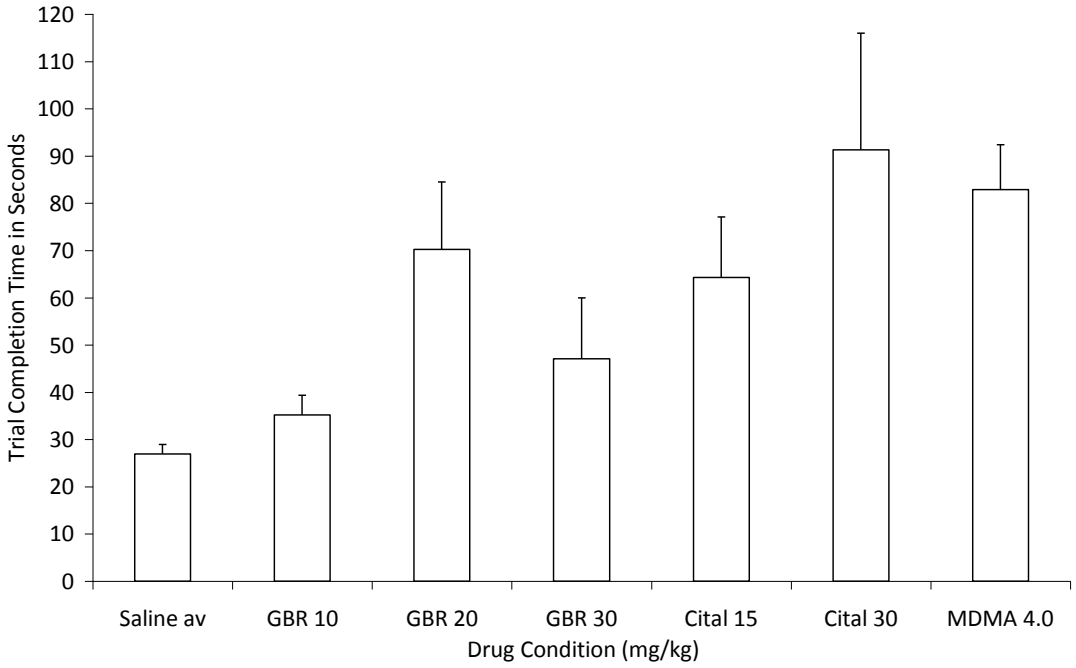

Figure 4: Average trial completion time in seconds across all rats for each drug and dose.

To examine the difference between drugs in terms of the error type the number of working memory errors made per session/day for each rat was obtained by adding together the number of working memory errors made in the three trials. These figures were then converted into percentage error values by taking the mean number of working memory errors and dividing by nine, the total number of working memory

errors possible. Reference memory errors per session/day for each rat were also calculated by summing the number of reference memory errors made across the three trials. These figures were then divided by twelve, as this was the maximum number of reference memory errors possible. This manipulation was done to take into account that a rat could not make as many working memory errors as reference memory errors and therefore proportional figures were more representative.

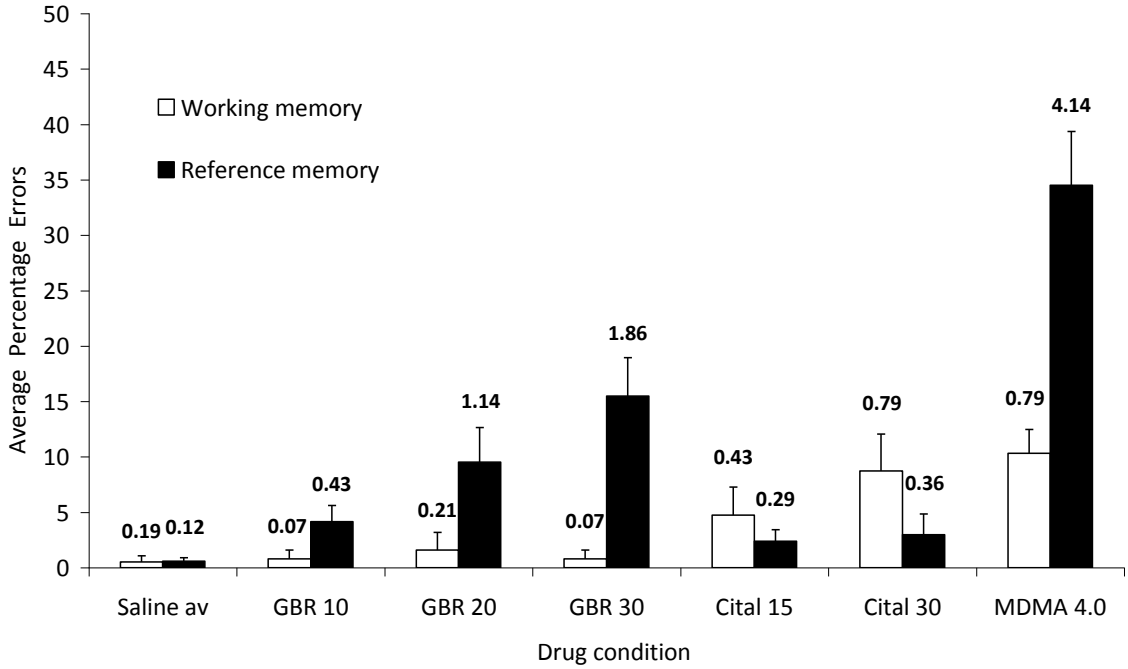

Figure 5: Average error percentage of working and reference memory errors across all rats for all drug doses. The values given above each bar are the mean number of total working or reference memory errors made in each condition across rats.

Group means for these data are presented in Figure 5 and show that saline produced very few errors of either type, while the 4.0 mg/kg dose of MDMA produced the most errors of both types. Also evident from Figure 5 both MDMA and GBR12909 produced more reference memory errors than working memory errors, however GBR12909 produced less reference memory errors than that seen with MDMA

administration. Citalopram in contrast, and as expected tended to produce more working memory errors than reference memory errors and the level of working memory errors was similar to that seen with MDMA administration.

Two 2-way repeated measures ANOVAs comparing error type and drug dose were used to analyse the data for GBR12909 and Citalopram. GBR12909 administration produced a significant main effect for error type, $F(1, 13) = 65.68$, $p < 0.05$ ($p = 0.00$) and a main effect for dose, $F(3, 39) = 6.79$, $p < 0.05$ ($p = 0.00$). There was also a significant interaction between error type and dose, $F(3, 39) = 6.01$, $p < 0.05$ ($p = 0.00$), showing that acute exposure to GBR12909 produced significantly more reference memory errors than working memory errors.

Citalopram administration failed to produce a main effect for error type, $F(1, 13) = 3.25$, $p > 0.05$, ($p = 0.10$). However, it did produce a significant main effect for drug dose, $F(2, 26) = 6.31$, $p < 0.05$ ($p = 0.01$). There was no interaction between error type and drug dose, $F(2, 26) = 1.12$, $p > 0.05$ ($p = 0.34$), hence Citalopram administration did not result in a significant difference in the type of error made.

Finally a 2-way repeated measures ANOVA comparing error type with MDMA and saline administration was conducted. A main effect for error type was found, $F(1, 13) = 23.70$, $p < 0.05$ ($p = 0.00$) as well as a main effect for dose, $F(1, 13) = 67.37$, $p < 0.05$ ($p = 0.00$). There was also a significant interaction between error type and dose, $F(1, 13) = 24.69$, $p < 0.05$ ($p = 0.00$) indicating MDMA administration produced significantly more reference memory errors than working memory errors.

Discussion

Kay et al. (2009) found acute MDMA administration produced more reference memory errors than working memory errors in the radial arm maze. To reiterate, the aim of this study was to replicate the acute effects of MDMA on performance in the radial arm maze and examine which neurotransmitter system plays a role in producing the MDMA induced reference memory effect seen in the radial arm maze. As acute MDMA administration produces an increase in both 5-HT and dopamine activity this study administered acute doses of both a 5-HT agonist (Citalopram) and a dopamine agonist (GBR12909) to examine which neurotransmitter system may be responsible for the reference memory effect seen with MDMA exposure. The current study found administration of MDMA significantly reduced accuracy and increased trial completion time in the radial arm maze. Therefore MDMA significantly disrupted performance in a task used to assess memory function. In addition both Citalopram and GBR12909 significantly affected accuracy where as the dose of both drugs was increased there was a significant decrease in the percent of correct arm choices in the maze. Therefore both 5-HT and dopamine stimulation reduced accuracy in the maze task. All drugs had an effect on trial completion time with drug administration generally increasing the amount of time it took to complete a trial. Once again both 5-HT and dopamine stimulation produced a significant effect on this measure of performance in the maze task.

The current findings concur with the reviewed animal literature that found that 5-HT manipulation has impaired performance in terms of accuracy and or the amount of time it took subjects to perform tasks that assess memory function such as radial arm maze tasks, (Santucci et al., 1996; Winter & Petri, 1987; Egashira et al., 2006; Buhot et al., 1995;) passive avoidance tasks (Santucci et al, 1996; Luttgen et al., 2005;

Ahlander-Luttgen et al., 2003; Rowan et al., 1990), DMTS/DNMTS tasks (Warburton et al., 1997; Jansen & Andrews, 1994, Herremans et al., 1995), water maze tasks (Carli & Samanin, 1992; Carli et al., 1995; Luttgen et al., 2005; Ahlander-Luttgen et al., 2003; Kant et al., 1996; Rowan et al., 1990; Naghdi & Majlessi, 2000; Majlessi & Naghdi, 2002) and a three-way panel task (Ohno et al., 1993). Also our findings are in agreement with the reviewed human research that has shown 5-HT manipulation has produced deficits in an array of cognitive tasks (Wadsworth et al., 2005; Robbe & O'Hanlon, 1995; Lader et al., 1986; Luciana et al., 1998).

The current findings also correspond with the reviewed dopamine animal literature that indicates that dopamine manipulation produces impairments in accuracy and or the amount of time it takes subjects to perform various cognitive tasks such as radial arm maze tasks (Buresova & Bures, 1982; Beatty et al., 1984), avoidance tasks (Zarrindast et al., 1992), object recognition tasks (Chuhan & Taukulis, 2006), DMTS/DNMTS tasks (Wright & White, 2003; Branch & Dearing, 1982; Kesner et al., 1981; Baron & Wenger, 2001; Harper et al., 2005) as well as conditional position, incremental learning task, time sensitivity and motivation (Moyorga et al., 2000).In addition these findings concur with human studies that have found that altering dopamine function reduces performance on cognitive tasks (Breitenstein et al., 2006; Shohmay et al., 2006; Montoya et al., 2008).

Possibly the most notable finding from the current study was that administration of the dopamine agonist GBR12909 produced significantly more reference memory errors than working memory errors. Also when MDMA was administered in the current study it produced this same pattern of more reference memory errors than working memory errors.

When MDMA was administered to rats in Kay et al. (2009) they found a significant increase in working memory errors in the radial arm maze. However, the increase in reference memory errors was significantly greater than that of the working memory errors. Therefore as GBR12909 produced significantly more reference memory errors in the current study it suggests that the stimulation of the dopaminergic system produces the reference memory effect seen in the radial arm maze. While the increase in working memory errors with acute exposure to the 5-HT agonist Citalopram failed to reach significance it still may be indicative that 5-HT may be responsible for the increase in working memory errors seen with the Kay et al. (2009) study. However, the conclusion that MDMA induced dopamine stimulation is responsible for the reference memory impairments seen with acute MDMA administration is tentative and would need to be further examined by pre-treating subjects with dopamine and 5-HT antagonists before they were administered MDMA. If the stimulation of dopamine levels produced by administering acute MDMA was blocked by administering a dopamine antagonist and reference memory errors were significantly diminished this would provide additional support that the reference memory effect is due to an increase in dopamine levels.

Many of the reviewed studies failed to differentiate between working and reference memory, however of those that did make this distinction, the current findings are in agreement with Harper et al. (2005) who found that MDMA administration significantly reduced accuracy in a fashion that may be due to a reference memory. Harper et al. (2005) also found that several dopamine agonists produced the same pattern of impairment in the DMTS task as that of MDMA. This is consistent with our finding that the dopamine agonist GBR12909 reduced accuracy in the radial arm maze in a similar manner of impairment to that seen with MDMA administration.

Beatty et al. (1984) found that the dopamine agonist d-amphetamine produced an increase in both working and reference memory errors in the radial arm maze but only if a delay was introduced into the paradigm. While our study also found that a dopamine agonist did produce an increase in reference memory errors it did not produce a significant increase in working memory errors. Also unlike their study we did not have to introduce a delay into our procedure to obtain a significant decrease in accuracy in the maze. However it could be that the increase in trial completion time seen with drug administration may act like introducing a delay into the paradigm.

The finding that dopamine agonists may produce reference memory impairments concur with studies that have examined human performance in tasks that involve dopamine such as assessing source recognition and proactive interference (Montoya et al., 2008) and procedural memory tasks (Thomas et al., 1996; Thomas-Ollivier et al., 1999). However our findings conflict with those of Buhot et al. (1995) who found that a 5-HT agonist produced significantly more reference memory errors than working memory errors in the radial arm maze and Santucci et al. (1996) who found that there was a significant increase in both working and reference memory errors in the radial arm maze during the 5-HT release phase of p-chloroamphetamine.

Of the studies that specifically differentiated between working and reference memory and 5-HT function our findings are conflict with those that found that 5-HT stimulation produced a significant increase in working memory errors (Santucci et al., 1996; Ohno et al., 1993). Although the administration of the 5-HT agonist Citalopram failed to produce a significant increase in working memory errors we did find evidence that suggests that 5-HT did significantly disrupt accuracy in the radial arm maze and this to a certain extent may involve working memory.

While the findings of the current study provide evidence that the dopaminergic system may be responsible for producing the reference memory effect seen in the radial arm maze, it is important to note that the effect that we witnessed with acute exposure to GBR12909 was clearly not as strong as that seen with MDMA exposure. There may be several reasons as to why this occurred. One possible explanation is that in order to produce the same level of impairment as that seen with acute MDMA administration it is necessary to activate both serotonin and dopamine activity. It may be that there is an additive or synergistic effect to the dopaminergic and the serotonergic system when MDMA is administered.

In addition it has been found that there is a relationship between serotonin and dopamine release. For example the serotonin receptor 5-HT_{2C} seems to play a role in the control of dopaminergic functioning within the brain (DiMatteo, Cacchio, DiGiulio & Esposito, 2002). Colado et al. (2006) also reported that 5-HT_2 receptors enhance the dopamine release found with acute exposure to MDMA. Therefore, it may be that the effects of acute MDMA on each neurotransmitter system may be very difficult to differentiate as the two appear to interact.

Another explanation for the smaller reference memory effect with acute administration of GBR12909 compared to MDMA was that we did not use a large enough dose of GBR12909. It may be that a larger dose of GBR12909 could have produced more comparable results with that of MDMA. However, this was not performed due to the difficulties in getting the GBR12909 into solution. Also the GBR12909 did produce a significant effect on trial completion time indicating that the drug was affecting performance in the radial arm maze.

It could also be useful to examine whether a larger dose of Citalopram would produce a stronger effect on working memory errors in the radial arm maze. We only

examined the effects of two doses of this drug whereas we used three different doses of GBR129009. However, with the two doses of Citalopram that we did use we obtained a significant effect on trial completion time that was comparable with that of the acute MDMA treatment. Therefore it could be argued that the drug was definitely having some pharmacological effect which was evident on performance in the maze.

There are many different 5-HT receptors. 5-HT receptors have been divided into families and subtypes which are 5-$HT_{1A/1B/1D/1E/1F}$, 5-$HT_{2A/2B/2C}$, 5-HT3A/3B, 5-$HT_{4A/4B/4C/4D}$, 5-$HT_{5A/5B}$, 5-HT_6 and 5-$HT_{7A/7B/7C/7D}$ (Meneses, 1999). Therefore there are seven classes of 5-HT receptors each with its own distribution and function in the brain. This makes research challenging because there are several different ways to increase or decrease serotonin levels in the brain and therefore it can be difficult to tell which drugs act on which 5-HT receptors. It also is problematic in that it is difficult to ascertain which 5-HT receptors are involved in which behavioural functions. There is the possibility that Citalopram may be too general in its pharmacological agonist effects as there is a reasonable amount of evidence suggesting that it may specifically be the 5-HT_{1A} receptor that plays a pivotal role in memory processes.

For example the serotonin agonist 8-OH-DPAT impaired performance on the Morris Water Maze indicating that it affects spatial learning (Carli & Samanin, 1992). Similarly Carli et al. (1995) found acute administration of 8-OH-DPAT significantly impaired performance on a spatial memory task using the water maze paradigm. Kant et al. (1996) also examined the effects of serotonin agonists 8-OH-DPAT on performance using a modified Morris water maze. Stimulation of the 5-HT_{1A} receptors impaired performance leading Kant et al. (1996) to argue that serotonin and 5-HT_{1A} receptors in particular, seem to play an important role in memory and learning. In particular relevance to the current study Winter and Petti (1987) found 8-OH-DPAT produced significant decreases in efficiency in the radial arm maze. This may be of

importance as this drug is a 5-HT_{1A} agonist which may play a role in memory function due to the large number of receptors found in the hippocampus, an area which has been found to play a role particularly in spatial memory (Winter & Petti, 1987). Therefore one possible reason why Citalopram did not produce a significant effect on working memory errors is that Citalopram may not be specific enough in its action on 5-HT_{1A} receptors.

Serotonin may also play a role in reference memory to a certain extent as Buhot et al. (1995) found that administration of the 5-HT_{1B} agonist CP-93,129 produced more reference memory errors than working memory errors in the radial arm maze. Therefore, reference memory errors may not have been produced due to Citalopram not acting specifically on 5-HT_{1B} receptors. Buhot (1997) also argues that manipulating serotonin non-specifically is problematic because serotonin has so many functions that it may alter other processes and behaviours that will affect performance rather than affecting memory per se. She further argued that manipulating the whole serotonergic system in a global manner is also not ideal as it can produce interactions with other neurotransmitter systems. Buhot (1997) suggested activating specific serotonin receptors to produce more comprehensive knowledge of the relationship between brain structures and cognitive processes. Therefore, it may not be ideal using a general serotonin agonist such as Citalopram when examining the cognitive effects of serotonin activation in the radial arm maze.

It may be useful for future research to examine the effects of other dopamine and serotonin agonists. Both agonist drugs used in the current study were reuptake inhibitors that work by preventing the neurotransmitters being taken back up into the pre-synaptic membrane and hence allowing the neurotransmitter to stay active in the synapse longer (Meneses & Hong, 1995). Therefore, these drugs are termed indirect agonists as they do not directly affect the release of the neurotransmitters. It may be

that different results would be produced by using different types of agonists that operate differently for example although MDMA does work by inhibiting reuptake of serotonin it also produces serotonin release from the pre-synaptic membrane (Malberg & Bonson, 2001). Similarly in rats MDMA produces the release of dopamine from cerebral tissue (Colado et al., 2004).Therefore, MDMA does not necessarily function the same way as Citalopram and GBR12909 and may not be able to encompass all the pharmacological effects that acute administration of MDMA produces. It could be useful to use agonists that produce dopamine and serotonin release in a more similar manner to that of acute MDMA exposure to ascertain whether the same effects on working and reference memory would be produced as those seen with administration of Citalopram and GBR12909.

The methodology of the current study had some potential flaws. Unfortunately the counterbalancing procedure was not ideal as due to technical difficulties we did not observe a very strict counterbalancing regime. This was due to the difficulty in dissolving the GBR12909 into solution as well as the impracticality of having to mix more than one drug solution up per day. It could also be beneficial to repeat the doses of each drug we used to clarify our findings to control for any extraneous variables that may have been present in a particular testing session.

As the current study suggested that stimulating dopamine activity appears to impair reference memory performance it may be advantageous to examine this finding further as there are several dopamine receptor types. Future study could examine whether the reference memory effect in the radial arm maze is due to either D_1 or D_2 receptor agonists. It would also be beneficial to further support these findings by administering dopamine and serotonin antagonists along with MDMA administration to see if blocking the activation of the dopamine and serotonin systems can attenuate the reference memory effect seen in the radial arm maze. If indeed the administration

of a MDMA and a dopamine antagonist does indeed reduce the amount of reference memory errors than MDMA administration alone it could corroborate the role of dopamine activity in reference memory.

In conclusion the current study examined radial arm maze performance in a paradigm that differentiates between working and reference memory. This study replicated the findings of Kay et al. (2009) that found acute exposure to MDMA significantly reduced accuracy in the maze and also produced significantly more reference memory errors than working memory errors. In addition the current study discovered that acute administration of the serotonin agonist Citalopram significantly reduced accuracy. It also found that the acute administration of the dopamine agonist GBR12909 significantly reduced accuracy in the maze and specifically produced more reference memory errors than working memory errors.

Therefore the main finding of this study was that it appears that stimulation of dopaminergic activity may be responsible for the reference memory effect in the radial arm maze seen with acute exposure to MDMA. Therefore dopamine activity seems to play an important role in reference memory which involves a long-term form of memory that entails the learning of task rules. However it is still unclear as to the underlying causes of this reference memory effect. It could be due to a long term memory retrieval problem, an impairment or confusion with the rules of the memory task or the result of proactive interference.

Study 2: D_1 and D_2 Agonists in the Radial Arm Maze

While many studies have found evidence that dopamine plays an important role in motor function and motivation the actual function that dopamine participates in with respect to memory processes is less clear (Bushnell & Levin, 1993). To make matters more complicated there are several different subtypes of dopamine receptor which are D_1, D_2, D_3, D_4 and D_5 (Vallone et al., 2000). These five receptor types are divided into two main subclasses which are D_1-like and D_2-like receptors (Jaber et al., 1996). These divisions are made on the basis of biochemical and pharmacological properties (Vallone et al., 2000). The D_1-like family of dopamine receptors include the D_1 and D_5 receptors while the D_2-like family consists of the D_2, D_3 and D_4 receptors (Vallone et al., 2000). The number of dopamine receptors that exist makes it difficult to determine what role each dopamine receptor plays in cognition.

There is some evidence that different behaviours are associated with different dopamine receptors. For example the D_2-like family have been related to the psychological disorder schizophrenia (Farde, 1997) and the emotional high associated with the use of stimulant drugs of abuse (Liechti & Vollenweider, 2001). Whereas the D_1-like family have been associated with locomotion (Jaber et al., 1996) and executive function, which involves learning and memory (Roesch-Ely et al., 2005). However there is also some evidence that D_2 receptors may play an important role in cognition. For example while there are a large number of D_1 receptors in important cognitive areas like the prefrontal cortex (PFC) there are also some D_2 receptors in this area (Muller, von Cramon & Pollmann, 1998). In addition there are both D_1 and D_2 receptors found in the hippocampus which is an area of the brain that is important in memory function (Umegaki et al., 2001). It is also thought that D_2 receptors may play

a role in working memory based on the findings that administering D_2 agonists alter memory performance possibly via the striatum (Ellis et al., 2005). However, it is not fully known whether the different dopamine receptors are involved in different roles of cognitive processing (Levin & Bowman, 1986). Also it still remains uncertain as to whether stimulation of various dopamine receptors produces improvements or impairments in memory performance (Zarrindast et al., 1992).

It has been argued that some cognitive functions such as working memory operate within an optimal level of dopamine activity where either too much or too little dopamine activity produces impairment (Williams & Castner, 2006). Thereby it is possible to examine dopamine receptor function by utilising either dopamine antagonists or agonists. As Study 1 found dopamine manipulation appeared to be produce the reference memory impairment seen in the radial arm maze task, the current study examined which dopamine receptor type might contribute to this reference memory impairment.

A common method of examining what roles different dopamine receptors play in cognition involves administering various dopamine receptor agonists or antagonists before subjects are tested on various cognitive tasks. Unfortunately for the purpose of the current study many of the tasks that are used to assess memory and cognition are confounded as they measure both working and reference memory. Hence to review the role of D_1 and D_2 receptors in cognition is it necessary to look at tasks that claim to assess various cognitive functions including working memory as often these paradigms assess reference memory as well. The effects of D_1 and D_2 receptor manipulation on cognition are summarised in Table 4.

Table 4: Summary of research on the effects of D_1 and D_2 agonists and antagonists on cognition.

Author (date)	**Drug (dose)**	**Drug Action**	**Method of Delivery**	**Subjects/ Participants**	**Cognitive Task/s**	**Results/Memory Effects**
Levin & Bowman (1986)	Quinpirole (0.03 to 1.0 mg/kg)	D_2 Agonist	I.P. injection	Rats	Standard 8 arm radial maze (all arms baited)	Significant impairments in arm entries & latencies
Sawaguchi & Goldman-Rakic (1991)	SCH23390 (10 to 80 µg)	D_1 Antagonist	Injected into dorsolateral PFC	Rhesus monkeys	Delayed (1.5 to 6 seconds) working memory oculomotor task (DMTS using eye movement)	Both D_1 antagonists - increase in errors & latency
	SCH39166 (1 to 10 µg)	D_1 Antagonist				
	Raclopride (100 µg)	D_2 Antagonist				D_2 antagonist – no effect on performance

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Memory Effects
Zarrindast et al. (1992)	SKF38393 (2 to 16 mg/kg)	D_1 Agonist	I.P. injection 30 minutes before testing except for SCH23390 via S.C. injection	Albino mice	Active avoidance learning task using mild foot shock. Testing 24 hours later to measure retention (assessed long-term retrieval)	SKF38393 improved retention performance.
	Bromocriptine (4 to 32 mg/kg)	D_2 Agonist				Low doses of bromocriptine improved performance & high doses significantly impaired.
	Quinpirole (0.25 to 2 mg/kg)	D_2 Agonist				Quinpirole no significant effect.
	SCH23390 (0.025 to 0.1 mg/kg)	D_1 Antagonist				Low doses of SCH23390 significantly impaired performance & higher doses had no effect.
	Sulpiride (20 to 60 mg/kg)	D_2 Antagonist				Low doses of sulpiride significantly impaired performance & higher doses had no effect.
						SCH23390 pre-treatment reduced the improvements seen with SKF38393 but sulpiride pre-treatment had no effect.
						Sulpiride pre-treatment reduced the impairment produced by bromocriptine.
						Pre-treatment with SKF38393 before bromocriptine increased impairment suggesting both D_1 & D_2 receptors involved in task performance.

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Memory Effects
Bushnell & Levin (1993)	D-amphetamine (0.30 to 1.0 mg/kg)	General Dopamine agonist	I.P. injection	Rats	DNMTS trials (assessing spatial working memory)	Dopamine agonist - significant impairment in working memory
	SKF38393 (1.0, to 3.0 mg/kg)	D_1 Agonist			Visual discrimination trials (like DMTS task except correct answer was cued by a light – assessed reference memory)	D_1 agonist - no significant impairment in either task
	SCH23390 (0.010 to 0.024 mg/kg)	D_1 Antagonist				D_1 antagonist - no significant impairment in either task
	Quinpirole (0.010 to 0.056 mg/kg)	D_2 Agonist				D_2 agonist - significant impairment in working memory
	Raclopride (0.056 to 1.0 mg/kg)	D_2 Antagonist				D_2 antagonist - no significant impairment in either task

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Memory Effects
Sawaguchi & Goldman-Rakic (1994)	Haloperidol (10 to 100 µg)	General DA Antagonist	Injected into dorsolateral PFC	Rhesus monkeys	Delayed (1.5 to 6 seconds) working memory oculomotor task (DMTS using eye movement)	Haloperidol - significant decrease in accuracy & increase in latency
	SCH23390 (10 to 80 µg)	D_1 Antagonist				SCH23390 - significant decrease in accuracy & increase in latency
	SCH39166 (1 to 5 µg)	D_1 Antagonist				SCH39166 - significant decrease in accuracy & increase in latency
	Raclopride (100 µg)	D_2 Antagonist				Raclopride – no significant effect
	Sulpiride (50 to 100 µg)	D_2 Antagonist				Sulpiride – no significant effect
Arnsten et al. (1994)	SKF38393 (0.001 to 0.5 mg/kg)	D_1 Agonist	I.M. injections	Young healthy rhesus moneys	Delayed response testing (had to remember location of reinforcer over various delays – working memory)	SKF38393 - low doses produced significant improvement. Higher doses - significant impairment.
	Dihydrexidine (0.001 to 1.0 mg/kg)	D_1 Agonist		Young experimentally dopamine depleted monkeys		Dihydrexidine - significant improvement in young monkeys but impaired the majority of elderly monkeys.
	SCH23390 (0.001 to 0.1 mg/kg)	D_1 Antagonist		Elderly monkeys (natural dopamine depletion)		SCH23390 impaired performance in young monkeys - no effect on elderly monkeys.
						Pre-treatment with SCH23390 blocked the improvements & impairments produced by both D_1 agonists

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Memory Effects
Arnsten et al. (1995)	Quinpirole (0.0001 to 1.0 mg/kg) SCH23390 (0.0065 mg/kg) Raclopirde (0.001 to 0.2 mg/kg)	D_2 Agonist D_1 Antagonist D_2 Antagonist	I.M. injections 1 hour before testing	Young healthy rhesus monkeys Young experimentally DA depleted monkeys Elderly monkeys (natural DA depletion)	Delayed response testing (had to remember location of reinforcer over various delays – working memory)	Quinpirole - small doses significantly impaired young monkeys & higher doses improved performance. Quinpirole – no overall significant effect in elderly monkeys. Pretreatment with SCH23390 did not reverse impairments produced by low doses of quinpirole but did reverse improvements seen with high doses. Pretreatment with Raclopride reduced the impairments & improvements seen with quinpirole administration
Cai & Arnsten (1997)	A77636 (0.001 to 0.1 mg/kg) SKF81297 (0.001 to 0.1 mg/kg) SCH23390 (10 µg/kg)	D_1 Agonist D_1 Agonist D_1 Antagonist	I.M. injections 1 hour before testing	Elderly rhesus monkeys (natural DA depletion)	Delayed response testing (had to remember location of reinforcer over various delays – working memory)	Both D_1 agonists - lower doses significantly improved & higher doses significantly impaired. Pre-treatment with D_1 antagonist reversed the improvements & impairments for both agonists.

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Memory Effects
Luciana & Collins (1997)	Bromocriptine (1.25 & 2.5 mg)	D_2 Agonist	Orally	Healthy human volunteers	Spatial task – delayed matching to position task	Bromocriptine - improved spatial task performance.
	Haloperidol (3 mg)	D_2 Antagonist			Non-spatial task (object memory) – delayed matching to sample using geometric stimuli	Haloperidol - impaired spatial task performance.
						Neither drug affected performance on non-spatial task
Zahrt et al. (1997)	SKF81297 (0.01 & 0.1 µg)	D_1 Agonist	Cannulae infusion into PFC	Sprague-Dawley rats	Delayed alternation T-Maze task	D_1 agonist - increase in errors & no increase in latency
	SCH23390 (0.01 & 0.03 mg/kg)	D_1 Antagonist	I.P. injection			Pre-treatment with D_1 antagonist before the D_1 agonist – no impairment
Seamans et al. (1998)	SCH23390 (0.05 to 5 µg /µl)	D_1 Antagonist	Infusion into PFC	Rats	Delayed win-shift task using an 8 arm radial maze	D_1 antagonist - significantly more errors
	Sulpiride (0.05 to 5 µg /µl)	D_2 Antagonist				D_2 antagonist – no significant effect

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Memory Effects
Wilkerson & Levin (1999)	Quinpirole (1.1 to 10 µg/side)	D_2 Agonist	Local infusions into ventral hippocampus	Sprague-Dawley rats	Standard 8 arm radial maze (all arms baited)	Quinpirole – significant improvement
	Raclopride (0.19 to 1.67 µg/side)	D_2 Antagonist				Raclopride - significant impairment
	Dihydrexidine (1.1 to 10 µg/side)	D_1 Agonist				Dihydrexidine – no significant effect
	SCH23390 (0.19 to 1.67 µg/side)	D_1 Antagonist				SCH23390 – no significant effect
Druzin et al. (2000)	PPHT (0.004, 0.04 & 0.4 µg/1µl)	D_2 Agonist	Bilateral micro-infusions into PFC	Wistar rats	U-maze - DMTS task with 0 & 3 second delays. RE errors – incorrectly entering same arm as previous trial. AE errors – incorrectly entering alternate arm as previous trial.	PPHT - significantly impaired delayed trials (more RE errors than AE errors - perseveration impairment, possibly due to impaired executive functioning).
	Sulpiride (0.03, 0.3 & 3 µg/1µl)	D_2 Antagonist				Sulpiride - significantly improved delayed trials (more AE errors than RE errors)

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Memory Effects
Mehta et al. (2001)	Bromocriptine (1.25 mg)	D_2 Agonist	Orally	Healthy human volunteers	Reward–responsitivity task (assessed motivation)	No effect on motivation task
					Pattern recognition	No effect on pattern recognition.
					Spatial recognition	No effect on spatial recognition.
					Spatial memory span	Significant improvement in spatial span performance.
					Self-ordered spatial working memory task	No significant effect on spatial working memory performance.
					Tower of London task	No significant effect on planning.
					Probabilistic reversal task	Significant impairment in probabilistic reversal performance.
					Concurrent reversal task	No significant effect on concurrent reversal performance
Kozlov et al. (2001)	SKF38393 (1 nmol)	D_1 Agonist	Microinjections into medial frontal cortex	Wistar rats	Delayed alternation Y maze take	D_1 Agonist – short delays no effect. Longer delays – significant improvement.
	SCH23390 (1 nmol)	D_1 Antagonist				Short & long delays D_1 antagonist – significant impairment.
Floresco et al. (2001)	SKF81297 (0.05 to 0.20 µg /0.5 µl saline)	D_1 Agonist	Cannulae infusion into PFC	Rats	Delayed win-shift task using an 8 arm radial maze	30 minute delay - significant impairment & 12 hour delay – significant improvement.

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Memory Effects
Umegaki et al. (2001)	Raclopride (8 µg/kg)	D_2 Antagonist	Cannulae infusion into hippocampus	Fischer-344 rats	14 unit T-maze (used aversive foot shock to move through maze)	D_2 Antagonist - significantly more errors
	Quinpirole (8 µg/kg)	D_2 Agonist	Cannulae infusion into hippocampus			D_2 Agonist - on its own did not produce significant impairment
	Raclopride (8 µg/kg) & Quinpirole (0.5 mg/kg)		Pretreatment via I.P. injection			Pretreatment with D_2 agonist - ameliorated previous impairment
Liao et al. (2002)	SCH23390 (0.05 & 0.10 mg/kg)	D_1 Antagonist	I.P. injection 1 hour before testing	Wistar rats	Partially baited 8 arm radial maze:	SCH23390 & Haloperidol - significant increase in number of arms entered & time taken to complete a trial.
	Spiperone (0.05 & 0.10 mg/kg)	Selective D_2 Antagonist			Place task – 4 arms of maze consistently baited used extra maze cues. Assessed spatial memory.	Spiperone – no effect on performance
	Haloperidol (0.08 & 0.16 mg/kg)	Non-selective D_2 Antagonist			Cue task – 4 arms of maze baited that changed each trial & signalled using within maze cues. Assessed non-spatial memory.	Cue task - all drugs had no effect on the arms entered. All drugs increased time taken to complete a trial (significant motor impairment rather than memory deficit.)

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Memory Effects
Gibbs & D'Esposito (2005)	Bromocriptine (1.25 mg) fMRI scan	D_2 Agonist	Orally	Healthy Human Volunteers	Spatial working memory tasks - delayed matching to location, delayed nonmatching to location & delayed matching to object.	D_2 Agonist - significant impairment in all tasks fMRI - decrease in brain activity during encoding
Stuchlik & Vales (2006)	A77636 (0.1 to 1.0 mg/ml)	D_1 Agonist	IP injection 20 mins before testing	Long-Evans rats	Allothetic place avoidance task (placed on rotating circular platform & had to evade foot shock).	D_1 agonist - significantly less errors & increase in activity
	SCH23390 (0.02 & 0.05 mg/ml)	D_1 Antagonist				D_1 antagonist - significantly more errors & decrease in activity
Von Huben et al. (2006)	SCH23390 (3.2 to 5.6 µg/kg)	D_1 Antagonist	IM injections	Rhesus monkeys	Progressive ratio schedule task	Both drugs - reduction in responding.
					Bimanual motor skill task	Both drugs – impaired motor ability.
	Raclopride (10 to 56 µg/kg)	D_2 Antagonist			Rotating turntable task (motor coordination)	Both drugs - impaired coordination.
					Self-ordered spatial search task	Raclopride - impaired performance. SCH23390 – no significant effect.
					Visuo-spatial paired associates learning task	Raclopride – significant impairment. SCH23390 – no significant effect.

Author (date)	Drug (dose)	Drug Action	Method of Delivery	Subjects/ Participants	Cognitive Task/s	Results/Memory Effects
Lumme et al. (2007)	PET scan	Measuring D_2/D_3 receptor activity		Healthy human volunteers	Wisconsin card sorting task (executive functioning & abstract reasoning)	Errors were correlated with high D_2/D_3 receptor binding suggesting involvement in executive functioning.
Rinaldi et al. (2007)	SCH23390 (6.25, 12.5 & 50 ng)	D_1 Antagonist	Bilateral injections into PFC	CDI mice	Spatial recognition test	Both drugs – time spent visiting displaced & non-displaced objects were not significantly different (spatial memory impairment).
	Sulpiride (12.5, 50 & 100 ng)	D_2 Antagonist			Object recognition test	Both drugs – no effect on time spent visiting novel object (no object recognition impairment)
Boulougouris et al. (2009)	Quinpirole (0.1 & 0.3 mg/kg)	D_2 Agonist	I.P. injection 20 mins before testing	Lister Hooded rats	2 lever reversal task – one lever reinforced the other not. Once criterion reached levers were switched & ability to change behaviour was assessed.	Quinpirole - no significant effect on acquisition but significantly impaired reversal learning (perseverative errors).
	Raclopride (0.1 & 0.3 mg/kg)	D_2 Antagonist				Raclopride - significantly impaired acquisition but not reversal. Pre-treatment with raclopride attenutated impairments produced by quinpirole.

I.M. – intra-muscular injection I.P. – intraperitoneal PFC – prefrontal cortex S.C. – subcutaneous DA - dopamine

The Role of D_1 Receptors in Cognition: Evidence From Humans and Monkeys

Within the cortical structures of the primate brain the PFC has the highest concentration of dopamine (Sawaguchi & Goldman-Rakic, 1994). PFC dopamine depletion produces memory impairments in monkeys suggesting dopamine receptors are involved in memory processes (Sawaguchi & Goldman-Rakic, 1991). Within the PFC there is an abundance of D_1 receptors but low levels of D_2 receptors suggesting D_1 receptors may play a more pivotal role in mnemonic processes (Sawaguchi & Goldman-Rakic, 1991). There are no studies examining D_1 receptor manipulation in humans as there is no selective D_1 agonist that can be administered to human participants (Barch, 2004). Administering D_1 antagonists to Rhesus monkeys significantly impairs performance on working memory tasks while D_2 antagonists do not (Sawaguchi & Goldman-Rakic, 1991; Sawaguchi & Goldman-Rakic, 1994). Thus D_1 receptors in the PFC may have a more prominent role in working memory than D_2 receptors (Sawaguchi & Goldman-Rakic, 1994). As humans and monkeys age there is a noticeable loss of dopamine in the PFC (Arnsten, Cai, Murphy & Goldman-Rakic, 1994) and this correlates with a marked decrease in PFC cognitive functioning (Cai & Arnsten, 1997). Therefore researchers have used elderly monkeys to study the role that dopamine receptors play in cognition. Administering D_1 agonists has impaired cognitive performance in elderly monkeys suggesting D_1 activity may be involved in cognitive performance (Arnsten et al., 1994; Cai & Arnsten, 1997).

The Role of D_1 Receptors in Cognition: Evidence From Rats

The reviewed research in Table 4 has found evidence that altering D_1 activity sometimes improves cognitive performance (Stuchlik & Vales, 2006; Floresco &

Phillips, 2001). However the majority of animal research suggests that altering dopamine activity by administering D_1 agonists and antagonists impairs performance on cognitive tasks (Stuchlik & Vales, 2006; Zahrt, Taylor, Mathew & Arnsten, 1997; Seamans, Floresco & Phillips, 1998; Floresco & Phillips, 2001; Kozlov, Druzin, Kurzina & Malinina, 2001).

These cognitive impairments include deficits in spatial memory in an avoidance task (Stuchlik & Vales, 2006), impairments in spatial memory in the radial arm maze (Seamans et al., 1998; Floresco & Phillips, 2001), deficits in spatial working memory and the ability to learn an alternation rule in a T-maze (Zahrt et al., 1997) and a Y-maze (Kozlov et al., 2001). While T-maze and Y-maze tasks are often used to assess working memory they do contain the learning of a fixed rule that subjects need to alternate their arm choices and hence these tasks do contain a reference memory component (Frick et al., 1995) which appears to be disrupted by D_1 receptor manipulation.

Administration of D_1 agonists has produced perseverative responding indicative of an impairment in PFC functioning (Zahrt et al., 1997). Perseverative responding is a pattern of impairment that has also been found with acute MDMA exposure in rats (Frederick & Paule, 1997) and may be the result of an impairment in understanding task rules indicating a reference memory type impairment. In addition manipulation of D_1 receptor activity by administering D_1 antagonists has produced evidence of proactive interference (Kozlov et al., 2001). As it has been argued that the impairments produced by MDMA administration, which increases dopamine activity, could be the result of proactive interference (Harper et al, 2005 & 2006). It could be useful to further examine this phenomenon using dopamine agonists that increase dopamine activity rather than antagonists which decrease it. In addition Zahrt et al. (1997) found the memory impairment produced by the administration of D_1 agonists

was attenuated with the pre-treatment of D_1 antagonists (Zahrt et al., 1997) suggesting the effects were due to D_1 receptor manipulation and not general drug effects. In conclusion researchers have argued that there is an optimal level of D_1 receptor activity for successful cognitive functioning where either over stimulation or inadequate stimulation of D_1 receptors seems to impair cognitive processing (Cai & Arnsten, 1997). However it is not clear whether working or reference memory processes are more affected by manipulating D_1 receptor activity.

The Role of D_2 Receptors in Cognition: Evidence From Humans and Monkeys

Some researchers have argued that the role of D_1 receptors in cognitive functioning is well established (Lumme, Aalto, Ilonen, Nagren & Hietala, 2007) and the role that D_2 receptors play in cognition is unclear (Lumme et al., 2007). However others have argued D_2 receptors may play a key role in cognitive functioning (VonHuben et al., 2006) and more research on the role of D_2 receptors is needed (Luciana et al., 1997). Research using human participants has found manipulating D_2 receptor activity alters performance on spatial memory tasks (Luciana & Collins, 1997) and probabilistic reversal learning (Mehta, Swainson, Ogilvie, Sahakian & Robbins, 2001). This suggests stimulation of D_2 receptors may impair the learning of task rules indicating a reference memory deficit. Further evidence that dopamine plays an important role in cognition comes from imaging studies. Using PET scans Lumme et al. (2007) found errors produced while participants performed the Wisconsin Card Sorting Task were correlated with high D_2/D_3 receptor bindings. This indicates these receptors may play a role in rule formation suggesting an involvement in reference memory processing. In addition Gibbs and D'Esposito (2005) found a D_2 agonist significantly impaired spatial working memory performance and fMRI imaging

showed a decrease in brain activity during encoding which may account for the impairments. Manipulating D_2 receptor activity in rhesus monkeys has also impaired working memory (Arnsten, Cai, Steere & Goldman-Rakic, 1995; Von Huben et al. 2006), attention, reinforcer efficacy, motivation and associative memory (Von Huben et al., 2006). Therefore, Von Huben et al. (2006) suggested that D_2 receptors may play a more pivotal role than D_1 receptors in these processes.

The Role of D_2 Receptors in Cognition: Evidence From Rats

Manipulating dopamine activity by altering D_2 receptor activity has produced mixed results. There are some studies which have found that utilising D_2 receptor agonists and antagonists have had no affect or improved memory performance (Bushnell & Levin, 1993; Wilkerson & Levin, 1999; Druzin, Kurzina, Malinina & Kozlov, 2000; Umegaki et al., 2001; Boulougouris, Castane & Robbin, 2009). However the majority of research suggests that manipulating D_2 receptor activity has impaired performance on a variety of cognitive tasks (Levin & Bowman, 1986; Bushnell & Levin, 1993; Wilkerson & Levin, 1999; Druzin et al., 2000; Umegaki et al., 2001; Boulougouris, et al., 2009)

These impairments include deficits in several areas of cognition such as reversal learning (Boulougouris et al., 2009), spatial learning in a 14 unit T-maze (Umegaki et al., 2001) spatial memory and executive functioning in a DMTS U-maze task (Druzin et al., 2000), spatial working memory in the radial arm maze (Levin & Bowman, 1986; Wilkerson & Levin, 1999) and working memory in a DNMTS task (Bushnell & Levin, 1993). In addition D_2 receptor manipulation has also been shown to produce a perseverative pattern of responding in a reversal learning task (Boulougouris et al., 2009) and in a U-maze task (Druzin et al., 2000). Therefore it

would seem increasing D_2 receptor activity produces perseveration in rule based tasks indicative of a reference memory impairment which has also been found with acute MDMA administration (Frederick & Paule, 1997). Therefore manipulation of D_2 activity has been found to alter both reference and working memory processes.

One study that has examined the effects of dopamine manipulation on cognition that has specifically tried to differentiate between working and reference is that of Bushnell and Levin (1993). Visual discrimination trials were was used to assess reference memory whereas standard DNMTS trials were used to assess working memory. Administration of the general dopamine agonist d-amphetamine impaired performance on working memory trials. D_1 receptor manipulation produced no effect on either task whereas administration of the D_2 agonist quinpirole significantly reduced accuracy on working memory trials. This suggests D_2 receptors play a role in working memory. While Bushnell and Levin (1993) argued reference memory processes were not affected the DNMTS task still involved a reference memory component. In addition it could be argued that the visual discrimination trials were easier and hence visual discrimination performance would be more difficult to disrupt.

Pre-treatment with D_2 antagonists has attenuated impairments produced by D_2 agonists (Boulougouris et al., 2009). In addition impairments produced by administering a D_2 antagonist into the hippocampus was ameliorated by pre-treatment with a D_2 agonist indicating manipulation of hippocampal D_2 receptor activity was responsible for the observed deficits (Umegaki et al., 2001) and not general drug effects. In conclusion there is strong evidence that D_2 receptors are involved in cognitive functioning. However whether D_2 receptors are more involved in working or reference memory processes remains unclear.

Contradicting Findings for D_1 and/or D_2 Receptors Producing Cognitive Deficits

Not all studies that have tried to differentiate between the roles of D_1 and D_2 receptors in cognition have found such clear cut results. There are many studies with conflicting findings or studies which have suggested both D_1 and D_2 receptors may be important in cognition. For example Rinaldi, Mandilo, Oliverio and Mele (2007) examined the effects of dopamine manipulation on memory performance. Administering D_1 and D_2 antagonists impaired performance on a spatial memory task while leaving object recognition intact. Therefore, dopamine manipulation did not alter object recognition memory and both D_1 and D_2 receptors may be involved in spatial memory.

Using a partially baited eight arm radial maze Liao, Lai and Lin (2002) examined the effects of D_1 and D_2 receptors on memory. A selective D_1 antagonist and a non selective D_2 antagonist significantly impaired spatial memory. However a selective D_2 antagonist did not have any significant effect on performance. Therefore Liao et al. (2002) argued D_1 receptors appeared to play a more crucial role in spatial memory. Accuracy on a non-spatial memory task (cued task) was not impaired by any drug but did increase reaction time suggesting decreased motor activity rather than a memory deficit.

Zarrindast et al. (1992) conducted an extensive study investigating the effects of dopamine manipulation on an active avoidance task. To assess long-term retention 24 hours after mice were trained on the task they were administered various D_1 and D_2 agonists and antagonists. Low doses of the general dopamine agonist apomorphine improved performance while the highest dose impaired performance. Pre-treatment with the D_2 antagonist sulpiride reversed these effects indicating the involvement of D_2 receptor activity in retention functioning. However, pre-treatment with the D_1

antagonist SCH23390 also reversed the impairment seen with the highest dose of apomorphine indicating D_1 receptor involvement.

Administration of the D_2 agonist bromocriptine produced a similar pattern where low doses improved, and large doses impaired, retention. Pre-treatment with the D_2 antagonist sulpiride ameliorated the impairment produced by bromocriptine indicating the changes in performance were due to D_2 receptor activity. Conversely administering another D_2 agonist quinpirole did not significantly disrupt or improve performance at any dose. Also when the mice were pre-treated with the D_1 agonist SKF38393 and then given bromocriptine it increased the impairment seen with bromocriptine indicating both D_1 and D_2 activity can affect retention.

The D_1 agonist SKF38393 significantly improved retention performance at all doses used. Pre-treatment with the D_1 antagonist SCH23390 reduced this improvement while the pre-treatment with the D_2 antagonist sulpiride did not have any significant effect. This indicated D_1 receptor activity can also influence retention performance. Finally administration of low doses of the D_1 antagonist SCH23390 and the D_2 antagonist sulpiride on their own both significantly impaired performance suggesting that decreasing D_1 and D_2 receptor activity can disrupt retention. Therefore this study produced a rather entangled set of findings suggesting both D_1 and D_2 receptors may be important in retrieving information from long-term memory.

To try and make sense of the variability of the effects produced by the different drugs Zarrindast et al. (1992) suggested the mechanism in which the drugs manipulate neurotransmitter activity may be a factor in explaining the conflicting results. They argued post-synaptic manipulation of D_2 receptor activity appeared to impair performance while pre-synaptic D_2 or post-synaptic D_1 receptor manipulation appeared to improve performance. In addition the active avoidance task is used to assess long-

term retention of a fixed rule (moving into the safe chamber to avoid a foot shock). Therefore this paradigm may involve reference memory as the rule does not change across trials and by assessing functioning 24 hours later it involved long-term memory processes which may also involve reference memory. However this paradigm used motor activity to assess learning which is potentially confounding as dopamine manipulation can alter motor activity and hence any effect could be due to changes in motor activity and not memory per se.

Therefore the findings of the current literature review are equivocal as there are reports of activation of the D_1 family of dopamine receptors both improving and impairing memory function. Similarly there is evidence that stimulating the D_2 family of dopamine receptors can both improve and impair performance on memory tasks. In addition there is evidence suggesting both D_1 and D_2 manipulation are important in cognitive functioning and reports that neither D_1 nor D_2 receptors have any effect on certain paradigms. Therefore it is difficult to say with any certainty what role the different dopamine receptors play in cognition.

In addition numerous tasks that have been used to assess memory performance claim to specifically assess working memory. However many of these paradigms could also assess reference memory as they involve the learning of a rule such as alternating arm or lever choices, or not entering arms of a maze that do not contain reinforcement, or matching to sample. These are long-term rules that do not change across trials and hence may involve a reference memory component. In addition the finding that both D_1 (Kozlov et al., 2001) and D_2 (Druzin et al., 2000) receptor manipulation can produce proactive interference may suggest that changing dopamine levels can induce reference memory impairments in terms of producing deficits in the understanding of task rules.

The Current Study

Study 1 found the dopamine agonist GBR12909 produced more reference memory errors than working memory errors in a partially baited radial maze. Hence the current study further examined the involvement of dopamine in this paradigm by administering both D_1 and D_2 agonists to examine which dopamine receptors contribute to reference memory functioning by measuring which type of errors are produced by the different agonists. The current study utilised the D_1 agonist A68930, a full selective and potent D_1 agonist (DeNinno et al., 1991). It also used quinpirole a selective D_2 receptor agonist (Levin & Bowman, 1986).

Based on previous findings examining the effects of D_1 and D_2 agonists it is difficult to conclude which dopamine receptor plays a more pivotal role in memory functioning. There is evidence that cognitive performance can be impaired when either D_1 or D_2 receptor activity is stimulated or reduced. Therefore it is difficult to predict which dopamine receptor will play a more important role in reference memory. However due to the clear evidence that dopamine manipulation can effect cognitive performance we hypothesise that either D_1 or D_2 stimulation will impair radial arm maze performance. In addition due to GBR12909 impairing reference memory in Study 1 we hypothesise that administering either the D_1 or D_2 agonist will produce a significant reference memory impairment.

Method

Subjects

This study utilised the subjects from Study 1 (14 white male Sprague-Dawley rats that were now sixteen to seventeen months old). Half way through this study one

rat died and hence only the conditions it produced data for were analysed while the remaining conditions involved the thirteen remaining rats.

As this study was carried out immediately after Study 1 the rats were already at criterion and did not receive any extra training. Again they were kept at approximately 85-90% (between 233 and 281 grams) of their free feeding body weight. They had continuous access to water and were kept on a 12:12-hour light:dark cycle and were run during the dark phase of this cycle.

Apparatus/Materials

The aluminium maze previously described and chocolate chips were used as reinforcers contained in the Petri dishes previously described in the general method section. A digital stopwatch was used to record the amount of time it took a rat to complete a trial. Saline 0.9 % was used to obtain a baseline measure to compare the other drug doses with. Drugs used were Quinpirole 0.04, 0.08 and 0.12 mg/kg and A68930 0.1, 0.3 and 0.9 mg/kg. Each drug was prepared on the day of use by dissolving to the required dose in 0.9 % saline solution.

Procedure

This study utilised a within-subjects experimental design with each rat receiving all drug types and doses. The maze running procedure was identical to the training phase during the drug sessions. All drugs were administered via an intraperitoneal (i.p.) injection twenty minutes before running. As in the previous study, rats were run in batches where the first four rats were injected and then fifteen minutes after the first rat was injected all four rats were run. Once this batch had completed running the maze the second batch was run and so on until all the rats were finished. A

drug session was conducted within a day and there were at least three days between drug sessions to prevent any lasting effects of the drugs from previous days.

Unfortunately due to the drug A68930 taking such a long time to arrive from suppliers this study was unable to be counterbalanced. Therefore, in this study all rats received the same dose of the drug being examined during a session. The first drug session involved all rats receiving 0.9 % of saline. During the second session all rats were administered a 0.04 mg/kg dose of Quinpirole. In the third session all rats received 0.08 mg/kg of Quinpirole and in the fourth session they were given the 0.12 mg/kg dose of Quinpirole. During the fifth session all rats were administered 0.1 mg/kg of A68930 and on the seventh sixth they were given 0.3 mg/kg of this drug. Session seven involved all rats receiving another dose of 0.9 % saline solution. While during the eighth session all rats were given 0.9 mg/kg of A68930.

Finally on the last day of the study the medium sized doses of both Quinpirole (0.08 mg/kg) and A68930 (0.3 mg/kg) were combined to examine if there were synergistic or additive drug effects. This was also done to see if both D_1 and D_2 receptors were important in producing the reference memory effect seen with acute MDMA administration. Another dose of MDMA 4.0 mg/kg was not administered in this study therefore the MDMA data from the previous study was added in to the analyses to compare the drugs in this study with.

Results

In all figure error bars show standard error of the mean. Percent correct figures were calculated in the same way as Study 1. These data are presented in Figure 6. The MDMA data is from the previous study (Study 1) to be used as a comparison to the

other drugs. Figure 6 clearly shows that for both Quinpirole and A68930 as drug dose increased percent correct decreased indicating that both drugs had a detrimental effect on accuracy. It is also clear that neither drug on its own had as large an effect on accuracy as MDMA administration. However the combination of the two drugs produced a deficit in accuracy which was more similar to that of MDMA administration.

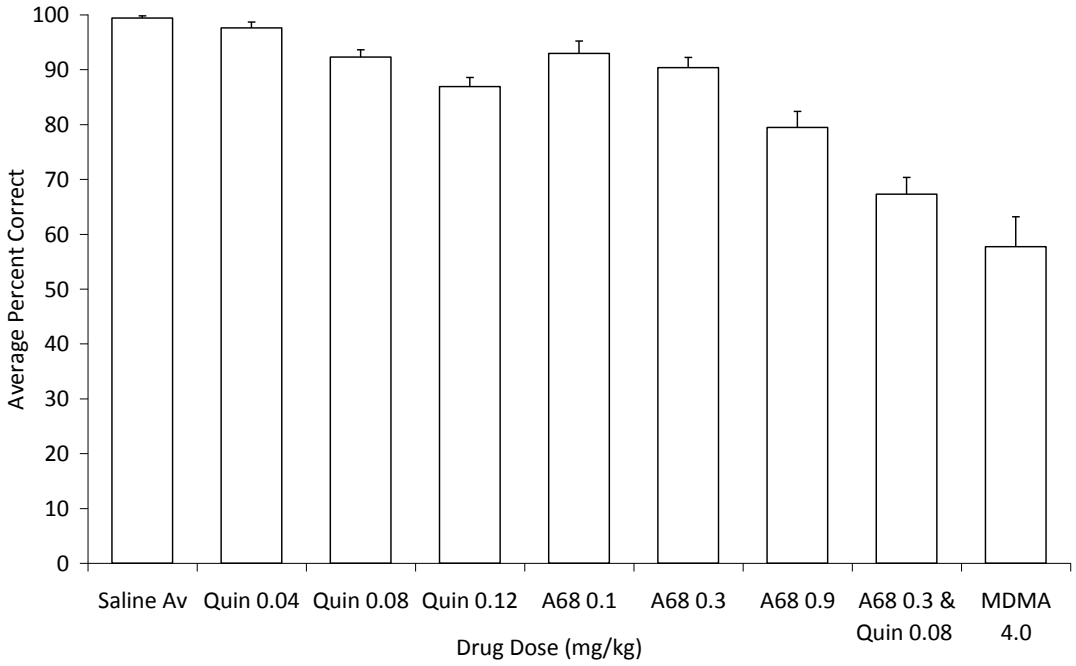

Figure 6: Average percent correct across all rats for each drug and dose.

A one-way repeated measures ANOVA comparing saline performance with that of Quinpirole revealed a significant effect for drug dose, $F(3, 39) = 19.91$, $p < 0.05$ ($p = 0.00$). There was also a significant effect for drug dose when comparing saline performance versus administration of A68930, $F(3, 36) = 20.22$, $p < 0.05$ ($p = 0.00$). Therefore as the drug dose of both the D_1 and D_2 agonists increased accuracy was significantly impaired. Finally a paired difference t-test revealed a significant

effect for saline performance versus the combined administration of A68930 0.3 mg/kg and Quinpirole 0.08 mg/kg, $t(12) = 10.43$, $p < 0.05$ ($p = 0.00$). Therefore the combination of the two drugs produced a significant impairment on accuracy compared to saline administration.

Average session trial completion times, in seconds, were calculated the same way as in the previous study. These data are depicted in Figure 7 and show that for both drugs mean trial completion times increased as drug dose increased compared to saline. Also the highest dose of each drug produced an increase in trial completion time similar to that seen with MDMA administration. A one-way ANOVA revealed a significant effect for saline performance versus Quinpirole administration, $F(3, 39) = 33.82$, $p < 0.05$ ($p = 0.00$).

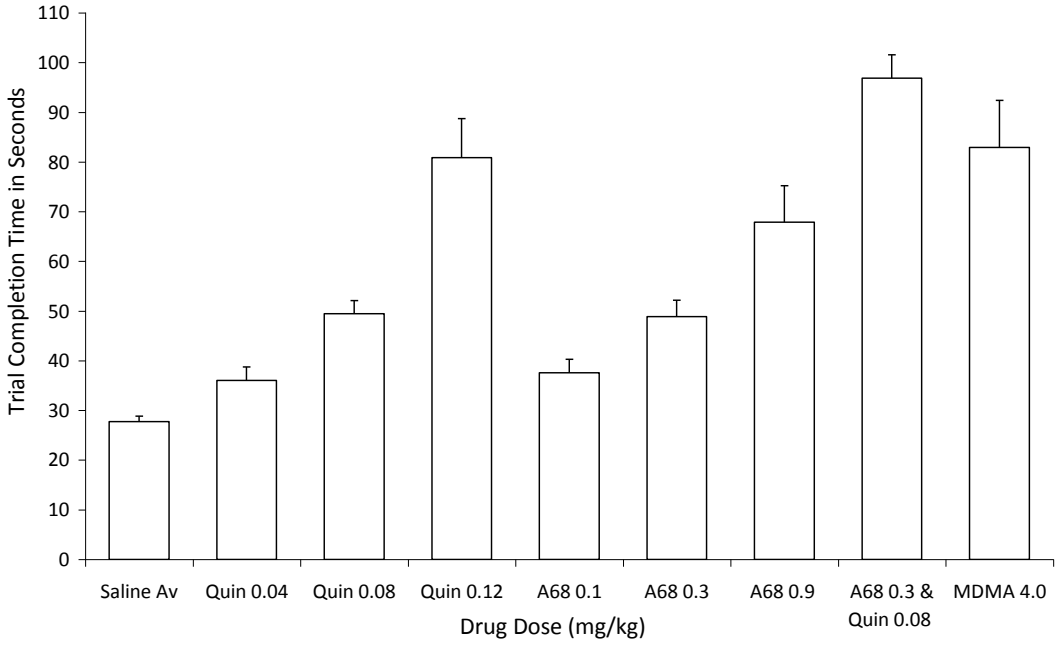

Figure 7: Average trial completion time in seconds across all rats for each drug and dose. Error bars show standard error of the mean.

There was also a significant effect for saline performance versus the administration of A68930, $F(3, 36) = 23.24$, $p < 0.05$ ($p = 0.00$). Therefore as the dose of both drugs increased the average amount of time it took for rats to complete a trial was significantly longer. Finally a paired samples t-test for saline versus the combination of Quinpirole 0.08 mg/kg and A68930 0.3 mg/kg revealed a significant difference, $t(12) = -14.05$, $p < 0.05$ ($p = 0.00$). Therefore when the two drugs were combined rats on average took significantly longer to complete a trial than when administered saline.

To examine the difference between error types the number of working memory errors made per session for each rat was obtained by adding together the number of working memory errors made in the three trials. Reference memory errors per session for each rat were also calculated by summing the number of reference memory errors made across the three trials. These figures were then converted into percentage error values in the same way as in Study 1. These data are presented in Figure 8 and show that the saline condition produced very few errors of either type while both Quinpirole and A68930 produced more reference memory errors than working memory errors. It should be noted that neither of these drugs, at least in the doses tested in the current study, on their own produced the amount of errors seen with MDMA administration. However, the combination of the medium sized doses of Quinpirole 0.08 mg/kg and A68930 0.3 mg/kg produced more reference memory errors than working memory errors and this impairment was similar in magnitude to that of MDMA.

A 2-way repeated measures ANOVA for error type versus drug dose for Quinpirole was conducted. It revealed a significant effect for error type, $F(1, 13) = 74.53$, $p < 0.05$ ($p = 0.00$) and a main effect for drug dose, $F(3, 39) = 19.92$, $p < 0.05$ ($p = 0.00$). There was also a significant interaction between error type and drug dose, $F(3, 39) = 10.21$, $p < 0.05$ ($p = 0.00$). These analyses were also conducted for A68930

and it also produced a main effect for error type, $F(1, 12) = 21.56$, $p < 0.05$ ($p = 0.00$) and drug dose, $F(3, 36) = 18.90$, $p < 0.05$ ($p = 0.00$). A68930 also produced a significant interaction between error type and drug dose, $F(3, 36) = 12.19$, $p < 0.05$ ($p = 0.00$). This indicates that both drugs produced significantly more reference memory errors than working memory errors.

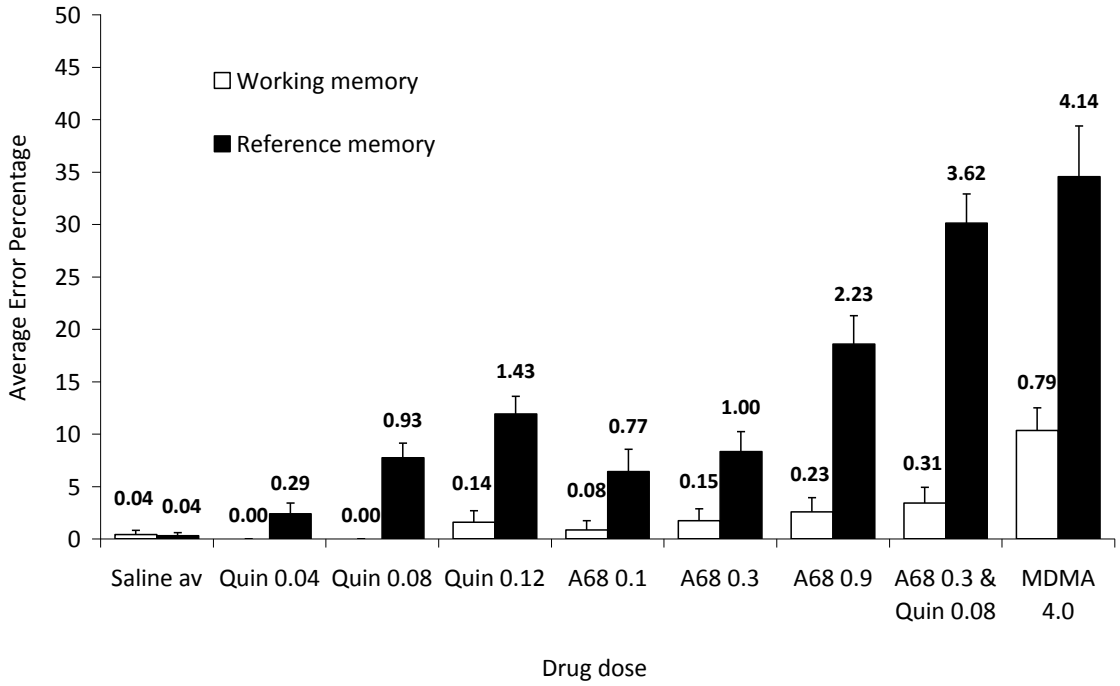

Figure 8: Average error percentage of working and reference memory errors across all rats for all drug doses. The values given above each bar are the mean number of total working or reference memory errors made in each condition across rats.

Finally a 2-way repeated measures ANOVA comparing error type and drug dose for saline versus the combination of 0.3 mg/kg A68930 and 0.08 mg/kg Quinpirole was conducted. It revealed a main effect for error type, $F(1, 12) = 75.74$, $p < 0.05$ ($p = 0.00$) and a main effect for drug dose, $F(1, 12) = 100.30$, $p < 0.05$ ($p = 0.00$). A significant interaction was also found, $F(1, 12) = 75.06$, $p < 0.05$ ($p = 0.00$),

indicating that the drug combination produced significantly more reference memory errors than working memory errors.

Discussion

The goal of the current study was to examine which dopamine receptor system was responsible for the reference memory effect seen in the previous study that found that administering the general dopamine agonist GBR12909 significantly decreased accuracy in a partially baited radial maze. Specifically the dopamine agonist produced significantly more reference memory errors than working memory errors. Therefore the current study administered the D_1 agonist A68930 and the D_2 agonist quinpirole to investigate which dopamine receptor may play a more crucial role in radial maze performance. The MDMA data from Study 1 was also presented in the results to allow comparisons between the degree of impairment seen with MDMA administration and the specific dopamine receptor agonists.

The current study found the D_1 agonist A68930 significantly impaired performance in the radial arm maze. As the dose of A68930 was increased accuracy significantly decreased and the average amount of time to complete a trial significantly increased. These findings are in agreement with Zahrt et al. (1997) who found the D_1 agonist also impaired memory performance using a maze task. However our findings contradict some of the previous research that has found that administering D_1 agonists actually improves memory performance (Stuchlik & Vales, 2006; Kozlov et al., 2001). In addition there are studies that have shown a more complicated dose related pattern where small doses of D_1 agonists have improved performance while larger doses have impaired performance on memory tasks (Floresco & Phillips, 2001; Arnsten et al.,

1994; Cai & Arnsten, 1997). The findings of current study produced a more simple dose dependent pattern of impairment where small doses produced small impairments while larger doses produced larger impairments.

The current study also found the D_2 agonist quinpirole significantly decreased accuracy in the radial arm maze as well as significantly increasing the average amount of time it took to complete a trial. These findings were also dose dependent and concur with those of Levin and Bowman (1986) who found Quinpirole significantly impaired performance in a radial maze task and Bushnell and Levin (1993) who found quinpirole produced deficits in a DMTS task. Similarly Druzin et al. (2001) found the D_2 agonist PPHT impaired memory performance utilising a U-maze paradigm and Gibbs and D'Esposition (2005) who found the D_2 agonist bromocriptine impaired memory performance in humans. However our findings are incongruent with some previous research that has found quinpirole had no effect on a complicated 14 unit T-maze paradigm (Umegaki et al., 2001) and actually improved performance in a radial arm maze task (Wilkerson & Levin, 1999). There is also some previous evidence of differing dose related patterns of behaviour with smaller doses of D_2 agonists improving performance and larger doses impairing performance (Arnsten et al., 1995). However in the current study Quinpirole only produced dose dependent impairments.

One possible explanation for the current study's dose dependent impairments could be due to high baseline levels of performance. There is evidence that suggests that the baseline level of performance is an important factor in the effects of dopamine manipulation (Barch, 2004). Administering dopaminergic agents when baseline performance levels are high tends to result in impairment, however when baseline levels are low dopaminergic agents may improve performance (Floresco & Magyar, 2006). Therefore as the rats in the current study achieved such a high stable level of

near perfect performance before drug administration it would be difficult to show that performance improved, resulting in a ceiling-like effect.

Interestingly the degree of impairment seen in terms of accuracy of arm choices with A68930 and quinpirole individually was much less than that seen with acute MDMA treatment. However, when two lower doses of the D_1 and D_2 agonists were co-administered they produced a more similar level of impairment to that seen with MDMA administration. In contrast to the results for accuracy both A68930 and quinpirole produced similar increases in trial completion time to that of acute MDMA exposure, in fact when the two lower doses of the drugs were combined they produced a trial completion time slightly greater than that of MDMA administration.

These findings are consistent with previous literature such as Rinaldi et al. (2007) who found that both D_1 and D_2 antagonists produced memory impairments suggesting that both types of dopamine receptor may play a role in memory function. However Rinaldi et al. (2007) administered antagonists which decrease dopamine activity whereas the current study used dopamine agonists that increase dopamine activity. This difference may produce difficulties in comparing the findings with the current study. In addition Rinaldi et al. (2007) did not examine the effects of co-administering the D_1 and D_2 antagonists but examined their effects individually which did not allow for additive or synergistic drug effects to be investigated.

In fact very few studies have co-administered D_1 and D_2 receptor agonists making the current findings of this study an interesting addition to the literature. One of the few existing studies that has examined this is Zarrindast et al. (1992) who found that co-administering the D_1 agonist SKF38393 with the D_2 agonist bromocriptine increased impairment produced by bromocriptine alone in an active avoidance task.

Therefore this finding suggested that both D_1 and D_2 receptors were involved in task performance and may produce an additive drug effect when combined.

The current findings conflict with many studies that have used drugs that manipulate both D_1 and D_2 receptor activity and found evidence that only one of the dopamine receptor types affect cognitive performance. For example our findings disagree with studies that have found that D_1 but not D_2 receptors are involved in cognitive functioning (Sawaguchi & Goldman-Rakic, 1991; Sawaguchi & Goldman-Rakic, 1994, Sawaguchi, 2001; Seamans et al., 1998; Muller et al., 1998). Our findings are also at odds with research that has found that D_2 but not D_1 receptor activity is involved in cognition (Bushnell & Levin, 1993).

Of most interest the current study found that when the D_1 agonist A68930 was administered significantly more reference memory errors were produced than working memory errors. Also when the D_2 agonist Quinpirole was given it also produced significantly more reference memory errors than working memory errors. However, when A68930 and Quinpirole were administered individually the level of reference memory impairment was much lower than produced by the acute dose of MDMA used in the previous study. When two lower doses of the D_1 and D_2 agonists were co-administered they produced a synergistic effect that resulted in a greater number of reference memory errors and so performance was more similar to that observed with the acute administration of MDMA. Therefore, it may be that both D_1 and D_2 receptors are involved in reference memory function in the radial arm maze.

Very few studies have utilised paradigms that specifically differentiate between working and reference memory. Bushnell and Levin (1993) used a task that contained visual discrimination (reference memory) and standard DMTS (working memory) trials to differentiate between the two types of memory processes. In contrast to the

current study they found administrating D_1 agonists and antagonists and a D_2 antagonist had no significant effect on either type of trial and a D_2 agonist produced a significant impairment only on working memory trials. Failure to find a reference memory effect in this study may be because the discrimination trials used to assess reference memory did not encompass the complexity of reference memory processes. During discrimination trials the rat simply had to push the lever with a light above it. The task may thus be viewed as more like an object recognition task which has been shown to be unaffected by dopamine manipulation (Luciana & Collins, 1997; Rinaldi et al., 2007). In addition this task appears much like the radial arm cued task used by Liao et al. (2002) that found no significant decrease in accuracy following the administration of D_1 and D_2 antagonists.

The finding that D_1 and D_2 receptors seem to interact where co-administering agonists produces either additive or synergistic effects is not a novel finding and has been reported in previous research. For example Robertson, Peterson and Worth (1992) found combining a D_1 agonist and D_2 agonist produced a synergistic effect that produced significantly more locomotor activity than either drug administered on its own. Unfortunately there seems to be a paucity of research on the effects of additive or synergistic actions of D_1 and D_2 receptors on cognitive functioning. However, Ichihara, Nabeshima and Kameyama (1992) found the combined administration of D_1 and D_2 agonists resulted in a synergistic effect on avoidance learning performance. Therefore the current study provides further evidence that D_1 and D_2 receptors may positively interact.

One difficulty in comparing research on the effects of D_1 and D_2 receptor activity on cognition is the differences in methodology used between studies. There are a number of factors that vary between the studies reviewed such as differences in the types of memory task used to assess cognition and the type and dosage of the

dopaminergic drugs administered. A further major difference in the literature is the method of drug administration. In many studies the agonists and antagonists have been administered directly into brain regions via cannula infusion but others have injected the drugs via intra-muscular, sub-cutaneous or intraperitoneal injections. These differences in methodology could produce conflicting findings as they will result in varying quantities of drug reaching the brain and therefore may produce different behavioural outcomes.

Another possible explanation for the conflicting findings in dopamine receptor research is the mechanisms by which different agonists and antagonists work. For example there have been some suggestions that where the drug takes effect within the synapse may be of importance. Zarrindast et al. (1992) argued that whether the drugs had different effects either via pre-synaptic or post-synaptic action could explain the conflicting findings as they found that stimulating post-synaptic D_2 receptors impaired memory retrieval whereas pre-synaptic D_2 stimulation and post-synaptic D_1 stimulation produced an improvement in memory function. However this explanation does not seem adequate as other researchers have found opposing findings such as Arnsten et al. (1995) who argued their findings suggested that low doses of quinpirole produced action at D_2 pre-synaptic autoreceptors that resulted in impairments in cognitive functioning. Further research into the effects of pre-synaptic and post-synaptic drug actions could be useful to elucidate the role of D_1 and D_2 receptors in cognition.

The difficulties experienced in the current study in trying to gain a comprehensive understanding of the function of D_1 and D_2 receptors are not surprising due to the complications involved. For instance it has been argued that trying to differentiate between the roles that D_1 and D_2 receptors play in cognition is not an easy task and is bound to be complex (Luciana et al., 1992). One possible reason for this is

that D_1 and D_2 receptors may work together and hence trying to differentiate between the two may prove difficult. For example Arnsten et al. (1995) argued that memory performance may involve interactions between D_1 and D_2 receptors. Floresco and Magyar (2006) argue that while D_1 receptors may play an important role in working memory it could be that D_1 and D_2 receptors may work together to influence behavioural flexibility such as that seen in paradigms like reversal learning and the Wisconsin card sorting task where subjects are required to change their behaviour in line with changing environmental conditions. Certainly the current study would suggest that both D_1 and D_2 receptors are involved in reference memory processes further highlighting evidence that dopamine activity may not easily be separated into clear differences in the roles of the varying receptor types.

There are also suggestions that that the two types of receptor may interact not only with each other but with other neurotransmitter systems (Luciana et al., 1992). For example Wilkerson and Levin (1999) argued D_2 receptors and acetylcholine may interact in the hippocampus to affect memory functioning. In addition the D_2 agonist Quinpirole produced an increase in acetylcholine release in the hippocampus (Umegaki et al., 2001). Moreover Hersi, Rowe, Gaudreau and Quirion (1995) found administering the D_1 agonist SKF38393 increased acetylcholine release in the hippocampus while the D_1 antagonist SCH23390 decreased it.

A possible reason for the reference memory impairment seen in the partially baited radial arm maze could be due to a long-term memory problem. It has been argued that D_1 receptors are crucial in memory retrieval (Liao et al., 2002) therefore it may be that administering D_1 and D_2 agonists impair the subjects ability to retrieve previously learnt information (the arms that contain reinforcement) from long-term storage. Similarly findings from avoidance procedures that test retention 24 hours after

training such as Zarrindast et al. (1992) provide further evidence that D_1 and D_2 receptor activity may produce impairments in long-term memory retrieval.

In addition the evidence from studies that have examined rule based learning such as reversal learning (Boulougouris et al., 2009; Mehta et al., 2001) and executive functioning card sorting tasks (Lumme et al., 2007) suggest that dopamine receptor activity may play a role in the reference memory deficit. Specifically, the impairment could be produced by subjects becoming confused as to the long-term rules involved in being able to perform various cognitive tasks.

An alternative explanation for the reference memory impairment seen in the radial arm maze could be due to proactive interference. For example Druzin et al. (2000) found a D_2 agonist produced evidence of proactive interference and perseverative responding. In addition Kozlov et al. (2001) also found evidence of proactive interference in their study that used both D_1 agonists and antagonists. These findings are in agreement with Harper et al. (2005) who administered MDMA and found evidence suggestive of a reference memory impairment and proactive interference. Therefore the reference memory impairment produced by MDMA in the radial arm maze may be due to both D_1 and D_2 receptor activity that produces proactive interference.

It would also be beneficial to examine other drugs that manipulate D_1 and D_2 receptor activity to try and gain further insight into the role that they play in cognition. Using agonists that operate in a variety of ways to increase D_1 and D_2 receptor activity may be useful in trying to disentangle the conflicting findings in this area of research. For example, examining whether direct or indirect agonists produce different findings and utilising various drugs that operate on different parts of the synapse may help clarify the underlying chemical cause of the reference memory impairments. To verify

the effects found in the current study it could be useful to replicate and extend the study by utilising various D_1 and D_2 antagonists.

Future research could pre–treat the subject with D_1 and D_2 antagonists and a combination of the two to examine whether they would attenuate the reference memory deficits produced by MDMA exposure alone. This could further support the current findings as if a combination of both D_1 and D_2 antagonists (blocking the increase in dopamine activity that MDMA produces) decreases the number of reference memory errors produced by MDMA, this would be further evidence for the role of D_1 and D_2 receptors in reference memory processes.

In conclusion the current study examined the effects of manipulating different dopamine receptors on performance in a partially baited radial arm maze that differentiated between working and reference memory. It was discovered that both A68930 and Quinpirole produced significant reductions in accuracy and increases in the amount of time it took to complete a trial. However these effects on their own were not as great as those seen with MDMA administration, but when two smaller doses of both D_1 and D_2 agonists were combined they produced findings similar to that seen with MDMA treatment. In addition both dopamine receptor agonists produced more reference memory errors than working memory errors and when smaller doses of the two drugs were co-administered they produced a synergistic drug effect that resulted in an impairment similar to that seen with MDMA administration. Therefore the current findings of this study would suggest that both D_1 and D_2 receptors are involved in reference memory. However as in Study 1 of the thesis it remains unclear as to the exact underlying nature of the reference memory effect. Further research is needed to clarify whether it is due to long-term memory problem, an impairment in terms of the long-term rules of the task or a product of proactive interference.

Acute MDMA Discussion

The literature review of the acute administration of MDMA strongly suggests MDMA exposure disrupts performance on several paradigms used to assess cognitive processes. In particular there are multiple studies indicating MDMA administration seems to impair reference memory performance (Kay et al., 2009; Harper et al., 2005; Braida et al., 2002). Therefore the first study of this thesis examined the acute effects of MDMA on memory performance by replicating the findings of Kay et al. (2009) who administered acute doses of MDMA to rats using a partially baited radial arm maze paradigm. They found MDMA exposure impaired maze performance producing a significant decrease in accuracy and a significant increase in the average amount of time taken to complete a trial. MDMA administration also produced a significant increase in working memory errors and reference memory errors; however the drug produced significantly more reference memory errors compared to working memory errors (Kay et al., 2009).

The first study of the current thesis utilised the same radial arm maze paradigm as that of Kay et al. (2009) using the dose of 4 mg/kg of MDMA that had the most pronounced effect in their study. The first study in the current thesis produced similar findings to Kay et al. (2009) as rats that were administered an acute dose of MDMA produced a significant decrease in accuracy as well as a significant increase in the average time it took to complete a trial. Study 1 also found that acute administration resulted in a significant increase in both working memory and reference memory errors. Finally of most interest MDMA exposure produced significantly more reference memory errors than working memory errors, thereby replicating Kay et al.'s (2009)

main finding that acute exposure to MDMA impairs reference memory more than working memory in the partially baited radial arm maze.

The finding that acute MDMA exposure produces a deficit in performance accuracy is consistent with previous acute MDMA research. For example LeSage et al. (1993) found that acute MDMA administration decreased both accuracy and response rates in a DMTS task. This impairment was characterised as a dose dependent impairment where higher doses of the drug produced more impairment than lower doses. Acute MDMA exposure also disrupted performance in a DNTMS task used by Marston et al. (1999). Both these tasks explained the impairments produced by MDMA exposure as the result of a short-term or working memory deficit. While the current findings of this thesis found that working memory processes were generally disrupted by MDMA exposure, it clearly showed that reference memory processes were more impaired by the drug. However it has been argued that DMTS and DNMTS tasks comprise a reference memory component in addition to working memory processes (Harper et al., 2005) and this may contribute to the findings that acute MDMA administration disrupts performance in these tasks.

Harper et al. (2005, 2006) also utilised a DMTS task and found MDMA and dopaminergic drugs resulted in delay-independent impairments in performance that are often attributed to attention or encoding deficits. However, on further analysis of their data they found evidence of proactive interference suggesting the deficits found with acute MDMA administration may be the result of a reference memory impairment whereby subjects become confused as to the rules of the task. This would be consistent with the results of the current thesis as it was found that acute MDMA administration produced more of a deficit in reference memory processes than working memory processes.

Using a standard "all-arms-baited" version of the radial maze Braida et al. (2002) found acute MDMA exposure significantly disrupted performance in a dose dependent manner that was attributed to a deficit in short-term memory. However, there was also some evidence of a reference memory impairment in that MDMA exposure disrupted arm entry patterns suggesting a rule like disruption in the strategies used for solving the task. This would concur with the current studies findings whereby MDMA administration produced both reference and working memory impairments. Unfortunately the classic radial maze paradigm utilised by Braida et al. (2002) does not allow unambiguous differentiation between working and reference memory processes as the procedure does not specifically measure both working and reference memory errors.

Taffe et al. (2001) used a number of tasks to assess the effect of acute MDMA exposure on cognitive performance. This involved DNMTS, spatial search, reaction time, motivation and bimanual motor tasks. Taffe et al. (2001) found acute MDMA administration produced significant impairments on performance across all cognitive tasks compared to that of the saline controls and attributed these impairments to a deficit in short-term memory. Therefore Taffe et al. (2001) found more of a general disruption in cognitive performance when subjects were administered acute MDMA treatment. These findings could also concur with those of the current thesis as many of the tasks that Taffe et al. (2001) utilised would contain both working and reference memory components which may account for the deficits found across such a range of cognitive tasks.

The current thesis findings are a little more complicated to compare with Frederick and Paule (1997) as they found mixed results when they administered an extensive battery of cognitive tasks (OTB) to monkeys who had received acute doses of MDMA. It was found that MDMA exposure produced significant disruptions to

time estimation, motivation and a sequence lever pressing task that changed each session. This sequence task utilised was designed to assess learning of a general task rule. It was found that under the influence of MDMA the monkeys made more acquisition (between session errors) than retention errors (within session errors). This type of deficit tends to suggest more of a reference memory impairment than a working memory impairment as they had more trouble learning a new task rule than remembering one which they had already acquired. This pattern of impairment is quite consistent with the findings from Study 1 in the current thesis.

However the current findings do not agree with all the previous research that has examined the effects of acute MDMA exposure on cognitive performance. Both Frederick et al. (1995) and Frederick and Paule (1997) used DMTS tasks and found no significant differences in performance when administering MDMA or saline to monkeys. A possible explanation for this may be the relatively low doses of MDMA used in these studies (0.1 to 1.0 mg/kg) relative to doses used in those that have found an effect.

Byrne et al. (2000) found no overall significant impairment in the acquisition of a lever pressing task with acute MDMA exposure. However the drug did significantly increase the latency to respond suggesting some degree of impairment or disruption in performance. Of note Byrne et al. (2000) examined cognition by investigating the effects of MDMA on the ability to acquire or learn a new task while the current thesis examined the effects of the drug on previously learnt task performance. It may be that acute MDMA administration affects these two cognitive processes differently explaining why Byrne et al. (2000) failed to find an effect of acute MDMA administration on performance.

As well as replicating Kay et al.'s (2009) findings with acute MDMA administration, the first study of this thesis also sought to examine which neurotransmitter systems may be responsible for the reference memory effect seen with MDMA exposure. As acute MDMA administration produces both an increase in 5-HT and dopamine activity the first study of this thesis investigated what role each of these neurotransmitter systems play in producing the reference memory impairment seen in the partially baited radial arm maze. Therefore the first study used the 5-HT agonist Citalopram and the dopamine agonist GBR12909 to examine whether either neurotransmitter plays a more pivotal role in reference and working memory errors in a partially baited radial arm maze. It was found that both agonists significantly reduced accuracy and increased the average time it took to complete a trial. Citalopram did not significantly affect one error type more than another. Conversely the dopamine agonist GBR12909 produced significantly more reference memory errors than working memory errors suggesting that alterations in dopamine activity may be more important in reference memory processes.

The second study of this thesis then examined which dopamine receptors play a more pivotal role in the reference memory impairment. Therefore the D_1 agonist A68930 and the D_2 agonist quinpirole were administered to examine their effect on performance in the partially baited radial maze. Both agonists produced a significant decrease in accuracy and an increase in the average time it took to complete a trial. Both agonists also produced significantly more reference memory errors than working memory errors. However, when administered individually neither agonist produced the level of impairment seen with acute MDMA administration. When the agonists were co-administered they produced a synergistic effect producing a deficit more similar to that seen with MDMA administration. Therefore it would appear that both D_1 and D_2 receptors play an important role in reference memory processes.

What Underlying Cognitive Processes Produce the Reference Memory Impairment?

There are a number of possible explanations for what is underlying the reference memory impairments seen with acute MDMA administration. An important issue involves trying to discern what sort of impaired cognitive processes are resulting in the reference memory errors as measured by the partially baited radial arm maze paradigm. One possible reason for the observed reference memory impairment is that acute MDMA exposure and acute dopamine manipulation induce a long-term memory impairment. This explanation suggests that rats have learnt which arms of the maze contain reinforcers, as during baseline and saline trials, performance is near perfect. However, after MDMA administration the rat's ability to retrieve this information from long-term memory becomes impaired. This disruption in long-term memory retrieval produces reference memory errors where the rats enter the unbaited arms of the maze as they can no longer access the information about which arms of the maze do contain reinforcement.

Support for this explanation may come from avoidance tasks that test long-term memory retrieval twenty four hours after initial learning. There is evidence to suggest that changes in dopamine activity can impair performance on these tasks (Zarrindast et al., 1992) indicating that altering dopamine levels can impair long-term memory retrieval which may account for the current findings in the radial arm maze. Also within the human Ecstasy literature Montgomery et al. (2005) found Ecstasy users were impaired in their ability to retrieve information from long-term memory using a word fluency task. Also Laws and Kokkalis (2007) conducted a meta-analysis of memory impairments in human Ecstasy research and found a stronger effect for long-term memory than short-term memory. However, this effect failed to reach significance but is suggestive that long-term memory may be slightly more affected by MDMA exposure than short-term memory. One possible reason why the rats make

more reference memory errors than working memory errors when their long-term memory becomes impaired is that when they are unsure of how to respond the rats rely on their innate predisposition to alternate rather than repeat arms (Chrobak & Napier, 1992).

Another possible explanation for the reference memory impairment in the partially baited radial arm maze is that acute MDMA administration and acute dopamine manipulation produce a disruption in the rules required to effectively perform a task. In other words MDMA exposure and dopamine manipulation produce problems in remembering what to do or how to perform when carrying out a task (Harper et al., 2005). Therefore the rats are able to remember where they have been (which arm they just entered) so do not tend to produce a large number of working memory errors. However they have become impaired as to what they should be doing within the task and have trouble remembering that they should be entering arms that contain reinforcement. Evidence from human Ecstasy use suggests that Ecstasy users have more trouble selecting a strategy to solve a cognitive task compared to drug free controls (Montgomery et al. 2005) which may be indicative of a reference memory type impairment.

Another reason for our findings that relates to the possibility of a deficit in task rules comes from Harper et al. (2005, 2006) who argued that acute MDMA exposure, along with various dopamine agonists, may produce proactive interference. This explanation may also account for the results seen in the radial arm maze where rats become impaired in entering the reinforced arms of the maze due to a confusion with the rules of the task.

An increase in proactive interference has also been found in studies that have manipulated dopamine activity (Harper et al., 2005, Druzin et al., 2000). This theory

postulates that due to proactive interference the subjects become confused as to which trial they are currently in. During the first two studies in the current thesis all rats received three trials a day and therefore if proactive interference occurred we would expect that performance should get worse across these trials. This is because the information from running the first trials may start to interfere with performance on the third trial. To examine this explanation further the data from the first two studies of the current thesis were re-analysed in a trial-by-trial basis. This enabled the average performance from trial one to be compared with the performance of trial two and finally performance on the third and last trial.

The data from Study 1 of the current thesis that examined performance after administration of acute MDMA, the 5-HT agonist Citalopram and the dopamine agonist GBR12909 are presented in Figure 9. There is no clear overall pattern to this data and there is definitely no evidence that performance worsens over trials. In fact for the MDMA data there is a slight tendency for performance to improve across trials although this effect is minimal. The data from Study 2 of the current thesis that examined the effects of administering the D_1 agonist A68930 and the D_2 agonist quinpirole on performance in the radial maze is presented in Figure 10. Again it clearly shows no obvious trends in the data in terms of trial by trial performance and definitely no clear evidence that performance worsened over trials.

Therefore it seems that explaining the current data in terms of a proactive interference effect is unlikely as the data from the two figures clearly show no evidence of a proactive interference like effect. However the explanation that the impairments in previous studies, such as Harper et al. (2005, 2006) that have found MDMA disrupts performance cannot be entirely ruled out. It could be argued that the three trials used in the current experiments are simply not enough for proactive interference to occur.

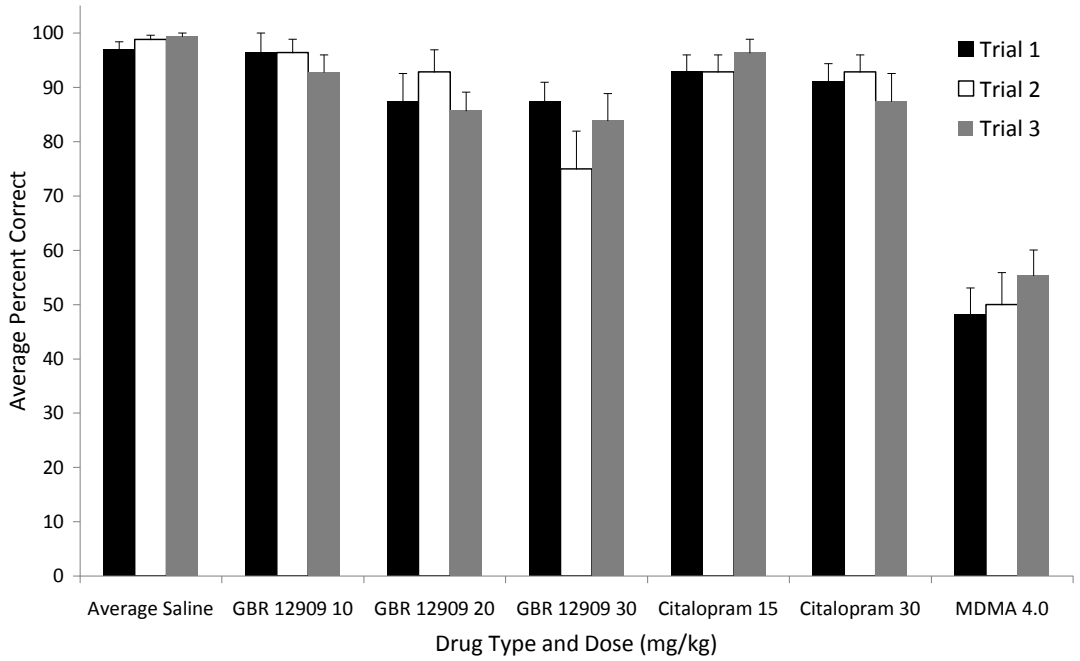

Figure 9: Average percent correct for different doses of GBR12909, Citalopram, saline and MDMA compared across the three trials that made up a testing session.

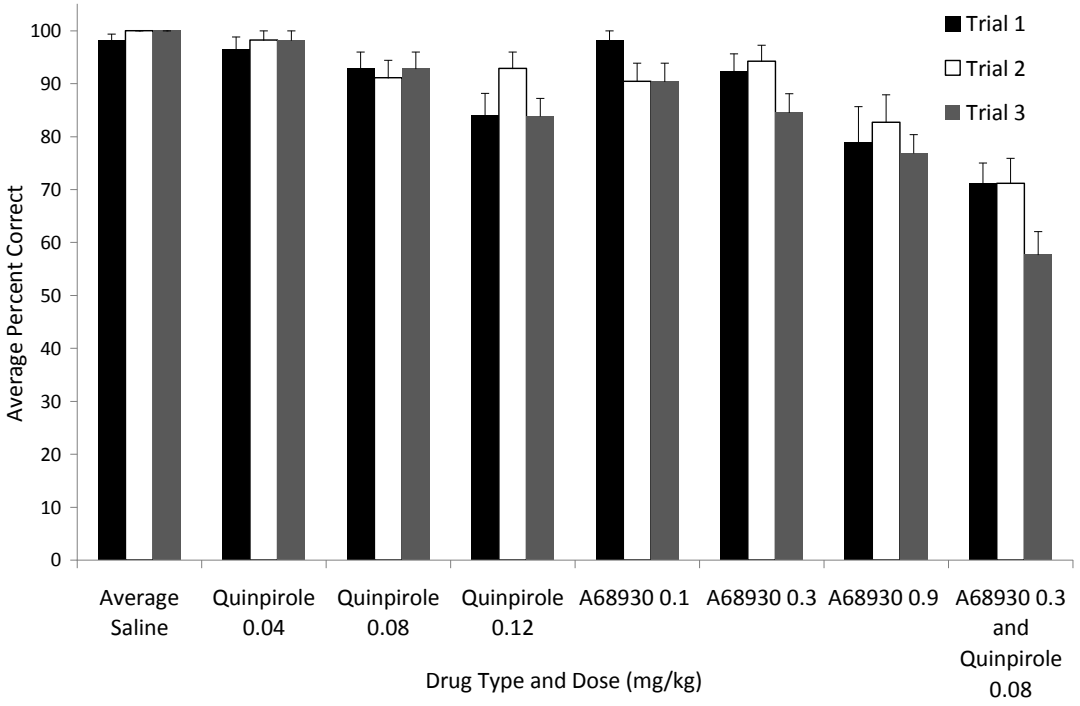

Figure 10: Average percent correct for different doses A68930, Quinpirole, saline compared across the three trials that made up a testing session.

While it is unlikely that proactive interference contributed to the findings of the studies within the current thesis it may contribute to research using DMTS and DNMTS tasks that use multiple trials within a testing session. It may be useful for future research to perform a partially baited radial maze task that utilises multiple trials per day to examine whether MDMA may produce proactive interference within the radial maze.

It could also be disputed as to whether a proactive interference explanation would be able to explain the reference memory effect seen within the partially baited radial maze. This is because if the rats are able to remember which arms contain reinforcement (the correct arm to enter), however they become impaired as to which trial they are in, then they would be more likely to go back and re-visit arms (at least as the start of the trial). A working memory error was classified as a rat re-entering an arm of the maze (either a baited one or an unbaited one) within a trial. Therefore if the rats remember the reinforced arms, but when given acute MDMA become confused as to where they are within the trials, they would be more likely to go back and re-enter these arms thus producing more working memory errors. Clearly this explanation then cannot account for the data in the current experiments due to the large number of reference memory errors made (entering an unbaited arm once within a trial).

Another possible explanation for the impairments seen in the radial maze may be due to perseverative responding. MDMA administration has been shown to produce perseveration which is characterised as persisting in a behaviour despite it no longer being effective (Head, Kennedy, Rodrigue & Raz, 2009). For example mice given MDMA produce perseverative locomotor activity where they engage in repetitive movement patterns (Powell et al., 2004). Frederick et al. (1995) and Frederick and Paule (1997) found acute MDMA administration produced perseverative responding in Rhesus monkeys in a tasks that involved learning sequences of lever pressing. There is

also evidence that Ecstasy users are impaired in tasks that assess executive functioning and one pattern of responding that has been produced is perseveration (Montgomery et al., 2005; Von Geusau et al., 2004). Evidence of perseverative responding has also been found in studies that have manipulated dopamine activity (Boulougouris et al., 2009; Druzin et al., 2000).

Hence this type of perseveration impairment could explain the current thesis findings that both MDMA and dopamine manipulation disrupt reference memory in the radial maze. Unfortunately it could also be argued that if the subjects are going to produce a perseverative pattern of responding then more working memory errors (repeating arms) than reference memory errors would occur. This is because a reference memory error is effectively going somewhere new, changing their previously learnt behaviour, while a working memory error would be an example of a repetitive behaviour.

Therefore there are several different explanations for what may produce the reference memory errors produced in the partially baited radial maze under the influence of MDMA. Unfortunately the findings from the current thesis are unable to conclusively verify which one of these particular explanations is most likely to account for the reference memory impairment seen with acute MDMA administration.

Pre-Synaptic Versus Post-Synaptic Receptor Activity and Acute Drug Administration

One possible explanation for the differences found between studies is the different places that a drug can act within the synapse. Different drugs can have either a pre-synaptic or a post-synaptic effect. There is research to suggest that where the drug has its effect within the synapse may determine the behavioural results. To make matters more complicated the same drug at different doses can also have either a pre-

synaptic or post-synaptic effect. Within the review of the dopamine and 5-HT research outlined in the introductions of the first two studies of the current thesis, there are some conflicting findings as to what happens to cognitive performance when various agonists are administered. One explanation for these differences is that the different agonists have different effects within the synapse.

For example Montoya et al. (2008) examined the effects of dopamine manipulation on a battery of cognitive tasks using the dopamine agonist apomorphine. They found at doses which primarily produce pre-synaptic effects apomorphine impaired performance on a variety of these tasks suggesting the pre-synaptic dopamine manipulation had a significant effect on cognitive performance. Further evidence of where a drug act on the synapse was found with Buresova and Bures (1982) who found that a pre-synaptic dose of the dopamine agonist amphetamine impaired radial arm maze performance. However Buresova and Bures (1982) found that a post-synaptic dose of apomorphine did not significantly affect performance. In addition Zarrindast et al. (1992) used many different D_1 and D_2 agonists and antagonists to examine memory retrieval in mice and found differences in the way that pre- and post-synaptic stimulation affected performance.

This effect has also been found within the 5-HT literature where Warburton et al. (1997) found evidence of a dissociation between the effects of stimulating pre- and post-synaptic 5-HT_{1A} receptors. Therefore there is evidence within the literature that suggests where a drug has its effect in the synapse may determine its effect on cognitive performance and this can vary by dose level. This is a factor that may contribute to the conflicting findings within dopamine manipulation research that could be used to explain the conflicting findings found with acute MDMA exposure. Evidence from the MDMA literature indicates that MDMA primarily works by releasing and inhibiting reuptake of 5-HT and dopamine pre-synaptically (Cole &

Sumnall, 2003). Further research could be conducted to examine this effect further and in particular examine if and how different doses of acute MDMA administration effects pre- and post-synaptic 5-HT and dopamine activity.

Alternative Explanations of the Acute Drug Administration Data

There are also some alternative explanations for the current thesis findings that do not directly relate to underlying memory processes. For example it may be that the deficits found in the radial maze after acute MDMA exposure are due to the drugs effects on motor function. Accuracy may be impaired not due to a disruption in cognitive processes but rather due to the drug producing a deficit in motor activity. In general when acute MDMA was administered to the rats in the current thesis a significant increase in the amount of time it took to complete a trial was produced. This may indicate that the rats had difficulty moving under the influence of MDMA. Anecdotally, when observing the rats, this did not seem to be the case as the increase in trial completion time seemed to be produced by spending more time circling in the middle of the maze. Once they did enter an arm of the maze they were able to run freely to the end of it. In other words they did not appear to show movement deficits rather an indecisiveness about which arms of the maze to enter. However, as we did not directly measure this we cannot exclude that part of the effect we observed may be due to motor activity impairments. Future research could use tracking techniques or photo beams at the beginning and ends of the maze arms which would enable the running speed of the rats to be calculated during saline and MDMA administration providing a more direct measure of motor ability.

In addition acute MDMA administration has been found to produce hyper-locomotion. For example Spanos and Yakamoto (1989) administered acute doses (2.5,

5.0 & 7.5 mg/kg) of MDMA to rats which produced a significant increase in activity counts in activity cages. Hence with doses comparable to those used in the current thesis MDMA has been shown to increase locomotor activity suggesting that the increase in trial completion times seen in the current thesis are unlikely to be the result of motor impairments causing the rats to move slower than normal.

It should also be noted from a theoretical perspective that if a motor impairment and hence slowing of trial completion time was producing the impairment in the radial maze, we might expect more working memory errors to be made than reference memory errors. This is because it has been shown within cognitive research that working memory processes are disrupted in a delay dependent manner (Dudchenko, 2004) in that the longer a subject has to retain information in working memory the more likely that information is to be impaired. Therefore, if a subject enters an arm of the maze and then has a long delay period it would be more likely to re-visit that arm as it would forget that it had already been there. However the current and previous research has found that MDMA administration produces more reference memory errors rather than working memory errors and therefore, the results of the current study may not be attributable to a motor impairment explanation.

A possible methodological explanation for the current findings is that the impairment seen with acute MDMA administration may be due to a disruption in motivational processes rather than cognitive processes. Both MDMA (Frederick & Paule, 1997; Nader, Hoffman & Barrett, 1989) and dopamine (Chuhan & Taukulis, 2006) agonists have been found to reduce the reinforcing value of food and reduce motivation to work for food reinforcers (Mayorga et al., 2000). Dopamine agonists have also been found to suppress the desire to eat (Wellman, Davis, Clifford, Rothman & Blough, 2009). Therefore one reason why rats entered non-reinforced arms is that they no longer valued the edible reinforcers that were available in the reinforced arms

of the maze. With no motivation to solve the task due to the decrease in the value of the food reward subjects may simply not care if arms they enter contain reinforcers or not. It could also be suggested that subjects produced more reference memory errors than working memory errors as again when performance is disrupted in some general way they may fall back on their natural disposition to alternate and not repeat behaviours (Chrobak & Napier, 1992). However subjects did tend to eat the reinforcers at the ends of the arm of the maze. Unfortunately this was not a measure that was recorded in the current thesis and therefore we cannot totally discount the reduction in the value of food reinforcers as a possible explanation for the current findings. Future research could include the number of reinforcers consumed as a useful measure of reinforcer value and another condition could involve pre-feeding the rats to examine what affect reducing food reinforcer value would have on performance.

It is also difficult to say with certainty that the impairments in reference memory performance seen with acute MDMA administration were the result of a memory deficit per se. Another possible explanation for the findings is that acute MDMA administration may produce attention deficits where the effects of the drug may have caused the subjects to no longer attend to the radial maze task. Although MDMA is chemically related to hallucinogens it has been argued that it does not produce the psychotic effects or hallucinations that hallucinogens can (Peroutka et al., 1988).

In human research visual distortions have been reported by Ecstasy users that take the form of luminescence of objects and flashes of light or objects in their peripheral vision (Peroutka et al., 1988). Thus it cannot be ruled out that non-human animals also experience such phenomena and a possible explanation for our findings is that the drug may have caused distracting visual distortions that disrupted the ability of the rats to attend to the task. This may be particularly important within the radial arm maze as there is evidence that subjects rely on extra-maze cues to solve the task (Liao

et al., 2002). Therefore acute MDMA administration may impair the rats ability to use extra-maze cues. Thus they can perform the task to a high level of accuracy during saline administration but when MDMA, 5-HT or dopamine agonists are administered it may affect their visual ability and attention processes rather than impairing memory processes. The argument that acute drug treatment produces visual impairments that impede the use of extra-maze cues is still problematic as there is research that suggests that it is the changes in 5-HT activity produced by acute MDMA administration that produces visual distortions and not dopamine activity (Liechti & Vollenweider, 2001). Therefore this explanation cannot explain the findings from the first two studies of the current thesis that suggest that it is the change in dopamine activity produced by acute MDMA administration that produces the reference memory effect in the partially baited radial maze.

There is no particular reason why this form of distraction would affect one type of memory error more than another and hence cannot fully explain our findings that MDMA affected reference memory performance more than working memory performance. Also, Harper et al. (2005) argued that MDMA did not produce attentional deficits in their study as they found that rats were influenced by how they had responded on a previous trial. For them to be influenced by what occurred on a previous trial they must have attended to it and this implied acute MDMA treatment did not impair attention. Although this task is not directly comparable to the paradigm of the current study it does suggest that the current thesis findings may not be due to a failure to attend to the task.

Future Research

To extend the current findings regarding acute MDMA, 5-HT and dopamine manipulation future research could utilise various antagonists to clarify the role that 5-HT and dopamine play in the reference memory impairment seen with acute MDMA exposure. Administering 5-HT and dopamine antagonists and then administering acute doses of MDMA could provide further support for our findings as if 5-HT or dopamine activity were blocked by the antagonists and subsequent MDMA exposure produced less reference memory errors this would be further evidence for the role of 5-HT or dopamine in reference memory processes.

It could also be interesting to examine whether acute MDMA administration would produce a difference in working memory and reference memory processes using a different paradigm to the partially baited radial maze. Unfortunately not many memory paradigms are equipped to assess both working and reference memory concurrently, however a holeboard task could possibly be used to achieve this. This paradigm involves an open field with 16 holes in its floor, a subset of which contain food reinforcers (van der Staay, 1999). Like the radial arm maze the holeboard is not rebaited within a trial, therefore if the subjects eats the reinforcers from one of the holes there is little point re-visiting that hole during a trial (van der Staay, 1999). Hence to perform efficiently within the holeboard task a subject has to keep track of which holes it has visited and quickly learn which of the holes contain food and which do not (van der Staay, 1999). Therefore like the partially baited radial maze it enables working and reference memory to be assessed. If a rat re-visits a hole within a trial it makes a working memory error and if it goes to a hole that does not contain reinforcement it commits a reference memory error (van der Staay, 1999). Thus this paradigm could be used to assess the acute effects of MDMA on working and reference memory and find further support for the findings of the current thesis.

Also as mentioned previously within this chapter it may be beneficial to examine the effects of acute MDMA exposure on proactive interference in the radial maze. Therefore more trials per training session would need to be conducted and possibly the manipulation of the inter-trial intervals could be conducted as that done previously by Harper et al. (2006) in a DMTS task. Finally to control for some of the potentially confounding variables within the current experiments some measure of motor activity and food reinforcer value should be included in future study.

Conclusion

In conclusion there is strong converging evidence that acute MDMA administration impairs reference memory processes. In particular using the partially baited radial maze paradigm acute MDMA administration produces more reference memory errors than working memory errors. In addition it appears that this reference memory impairment is due to changes in dopamine levels rather than alterations in 5-HT activity. More specifically this change in dopamine activity that disrupts reference memory processes appears to involve both D_1 and D_2 dopamine receptors. However, these conclusions are tentative as they would require further study where 5-HT and dopamine antagonists as well as D_1 and D_2 dopamine receptor antagonists would need to be administered before acute MDMA exposure to examine whether any of these manipulations would attenuate the reference memory effect. In addition the underlying cognitive mechanisms that produce this form of reference memory impairment are still unclear. Future research is required in order to differentiate between several explanations of the data including acute MDMA administration producing long-term memory deficits, impairments in tasks rules, proactive interference and perseverative responding.

PART TWO:

Chronic/Binge Effects of MDMA

Chronic MDMA and Cognition

There is considerable evidence that chronic Ecstasy use in humans has been associated with a range of cognitive impairments (see General Introduction for a review). These findings are confounded by a number of variables such as the purity of Ecstasy tablets, the amount used, pre-existing cognitive impairments, the self report measures used and polydrug use (Winsauer et al., 2002). These factors make determining whether MDMA actually causes cognitive impairments difficult and they also make establishing the exact nature of the cognitive impairments seen in Ecstasy users difficult to ascertain. Therefore animal studies are an ideal way of resolving some of these issues as they provide a much greater degree of experimental control (Taffe et al., 2002). However the animal studies that have examined the effects of chronic MDMA exposure on cognition have produced mixed results (Able, Gudelsky, Vorhees & Williams, 2006) and finding functional or behavioural impairments produced by chronic MDMA administration has not been easy (Winsauer et al., 2002).

Typically studies that utilise non-human animals examine the chronic effects of MDMA administration by administering chronic or toxic regimes of MDMA. These usually consist of multiple injections of MDMA given over a course of several days (Baggott & Mendelson, 2001). Chronic MDMA studies usually involve two main study designs. The first involves subjects being trained to perform a cognitive task until they reach a stable level of performance. A regime of MDMA is then administered and the subjects' performance on the previous task is re-assessed to

examine whether the MDMA produces disruption of an already learnt task. The other type of study examines acquisition where MDMA is administered and then after a period of time subjects are given a cognitive task to learn. Their performance is compared against that of saline controls to investigate whether there is any long-term impairment in their ability to acquire the task. The term chronic usually refers to long-term drug exposure that occurs over many sessions to examine the long-term effects of drug exposure. For example there are developmental MDMA studies such as Skelton et al. (2006) that administer MDMA to young rats every day for long periods (up to 10 days). Although some studies that have examined the effects of MDMA on cognition have used the term chronic to describe the regime of MDMA they use, typically these studies involve short courses of large doses of the drug. Hence technically they do not involve chronic drug exposure. In addition there has been some research that has referred to these regimes as toxic regimes. However, this term is now controversial as there is debate as to whether MDMA can be considered a neurotoxin (Bauman et al., 2007).

Therefore for the purpose of the current thesis the term binge will be used to refer to regimes of MDMA that involve typically larger doses than those used in research examining the acute effects of the drug, but are administered over either one or a few sessions and hence do not fit the definition of chronic drug exposure. In addition the term chronic regime will be reserved for studies that exposed subjects to at least ten sessions of drug exposure or involved intermittent exposure that lasted over several weeks. Many different kinds of tasks have been used to assess the effects of chronic/binge MDMA administration on cognition. Therefore this chapter will review the findings from studies that have utilised a number of different paradigms to assess the effects of chronic and binge MDMA exposure on cognitive functioning and these findings are summarised in Table 5.

Table 5: Summary of research on the binge and chronic effects of MDMA on cognition.

Author (date)	Drug regime (dose)	Method of Delivery	Subjects	Cognitive Task (s)	Results/Memory Effects
Slikker et al. (1989)	MDMA (1 injection of 5or 10 mg/kg for 4 days)	Oral gavage	Sprague-Dawley rats	Acquisition of 24-arm complex maze (training began 2 weeks after drug treatment)	No effect on performance/acquisition.
Li et al. (1989)	MDMA (2 x 6 mg/kg for 4 days)	S.C.	Sprague-Dawley rats	DRL Task (72 second inter-response interval – task acquired before drug treatment)	Binge regime had no effect on performance (subsequent acute challenge resulted in sensitisation).
LeSage et al. (1993)	MDMA (3.2 mg/kg per day for 20 days)	Injection (type not specified)	Pigeons	DMTS Task (0, 3, & 6 second delays, task acquired before drug treatment)	No difference in performance before and after chronic drug treatment (subsequent acute challenges resulted in tolerance).
Ricaurte et al. (1993)	MDMA (2 x 20 mg/kg per day for 4 days – repeated 1 week later).	S.C.	Long-Evans rats	Delayed Alteration in T-Maze (5 - 180 second delays, training began 7 weeks after drug treatment)	No significant effect in learning the task with 0 delay & no effect when delays added.
Robinson et al. (1993)	MDMA (8 x 10 mg/kg, 1 injection every 12 hours)	I.P.	Sprague-Dawley rats	Morris water maze acquisition (8 trials per day – platform shifted to new location each day, training began 2 days after drug treatment).	Initial impairment in search strategy when platform in new location but once found were able to find it on remaining trials – within 4 days of training performing at similar level to controls (transient impairment).

Author (date)	Drug regime (dose)	Method of Delivery	Subjects	Cognitive Task (s)	Results/Memory Effects
Frederick et al. (1995) & Frederick & Paule (1997)	MDMA (escalating doses of 2 x 0.10-20.0 mg/kg – each dose given for 14 consecutive days which took approx 4 months)	I.M.	Rhesus monkeys	DMTS Task (2 – 48 second delays) Incremental Repeated Acquisition Task Colour & Position Discrimination Task Time Estimation Task Progressive Ratio Task (All tasks acquired before drug treatment)	No difference in performance before and after chronic drug treatment (tolerance developed with repeated exposure).
Frederick et al. (1998)	MDMA (2 x 10 mg/kg for 4 days)	I.M.	Rhesus monkeys	DMTS Task (2 – 64 second delays) Incremental repeated acquisition task Colour & Position Discrimination Task Time Estimation Task Progressive Ratio Task (All tasks acquired before drug treatment)	No difference in performance before and after binge drug treatment (subsequent acute challenges resulted in tolerance).
Marston et al. (1999)	MDMA (2 x 10 mg/kg day 1) (2 x 15 mg/kg day 2) (2 x 20 mg/kg day 3)	I.P.	Lister Hooded rats	DNMTP Task (0.3 – 30.0 second delays, task acquired before drug treatment)	Delay dependent impairments during acute treatment & still present 16 days post drug-treatment indicating long-term working memory impairment (evidence of tolerance developing over the 3 days of acute drug treatment).

Author (date)	Drug regime (dose)	Method of Delivery	Subjects	Cognitive Task (s)	Results/Memory Effects
Byrne et al. (2000)	MDMA (2 x 20 mg/kg for 4 days)	S.C.	Sprague-Dawley rats	Acquisition of DRL Task (0, 10 & 20 second inter-response intervals, training began 2 weeks after drug treatment)	No effect on group performance – but 25% of MDMA treated rats could not learn the task with the 20 second inter-response interval.
Broening et al. (2001)	MDMA (2 x 5 mg/kg per day from P1-10 or P11-20) (2 x 10 mg/kg per day from P1-10 or P11-20) (2 x 15 mg/kg per day from P1-10 or P11-20) (2 x 20 mg/kg per day from P1-10 or P11-20).	S.C.	Sprague-Dawley rats	Straight channel swim (P60) Cincinnati water maze (P63) Morris water maze acquisition (P77) Morris water maze reversal (after acquisition phase) Morris water maze shifted/reduced platform (after reversal phase) Morris water maze cued learning (P70)	No effect on swimming speed. P1-10 treated rats no effect on performance on Cincinnati maze, Morris maze acquisition, Morris reversal or shifted/reduced platform. P11-20 treated rats significantly impaired on performance in Cincinnati maze, Morris maze acquisition, Morris maze reversal & shifted/reduced platform phase. Impaired memory probe trials (conducted at the end of each Morris water phase) for P11-20 rats but not P1-10 treated rats. No effect on cued learning in either group.
Morley et al. (2001)	MDMA (4 x 1 mg/kg for 2 days) (4 x 5 mg/kg for 2 days)	I.P.	Albino Wistar rats	Object recognition Task (15 & 60 minute delays, tested 14 weeks after drug administration)	Rats treated with higher dose of MDMA spent less time exploring novel object with the 15 minute delay but not the 60 minute delay (possibly due to floor effect) suggesting impaired non-spatial working memory.
Taffe et al. (2001)	MDMA (2 x 10 mg/kg for 4 days).	I.M.	Rhesus monkeys	DNMTS (0 – 64 second delays) Self Ordered Spatial Search (SOSS) Progressive Ratio Task (All tasks acquired before drug treatment)	Performance impaired on all tasks during drug treatment but returned to baseline levels after 1 week suggesting binge MDMA treatment had no long-term effect on performance.

Author (date)	Drug regime (dose)	Method of Delivery	Subjects	Cognitive Task (s)	Results/Memory Effects
Winsauer et al. (2002)	MDMA (2 x 2.5 mg/kg for 4 days). (18 days later 2 x 5 mg/kg for 4 days).	I.M.	Squirrel monkeys	Repeated Acquisition Task (Task acquired before drug treatment)	No effect on performance (no difference between baseline & either drug treatment) suggests chronic MDMA does not impair serial learning.
Sprague et al. (2003)	MDMA (2 x 20 mg/kg given 12 hours apart)	S.C.	Sprague-Dawley rats	Morris water maze acquisition followed by probe trials with platform removed (Training began 7 days post drug treatment)	No effect on learning but impaired performance on probe trials.
Williams et al. (2003)	MDMA (2 x 20 mg/kg per day from P11-20)	S.C.	Sprague-Dawley rats	Straight channel swim (P50) Cincinnati water maze (P51) Morris water maze acquisition (P57) Morris water maze reversal (P64) Morris water maze shifted/reduced (P71) Morris water maze cued learning (P78)	No effect on swimming speed. Impaired Cincinnati maze performance (longer latencies, more errors) Impaired performance on Morris maze acquisition, reversal & shifted/reduced performance phases. No effect on cued learning phase of Morris water maze.
Piper & Meyer (2004)	MDMA (2 x 10 mg/kg given every 5th day from P35-60).	S.C.	Sprague-Dawley rats	Object Recognition Task (15 minute delay, tested 5 days after drug treatment finished).	MDMA treated rats spent less time exploring novel object, suggests impaired non-spatial working memory.

Author (date)	Drug regime (dose)	Method of Delivery	Subjects	Cognitive Task (s)	Results/Memory Effects
Vorhees et al. (2004)	MDMA (2 x 5 mg/kg per day from P11-20) (2 x 10 mg/kg per day from P11-20) (2 x 20 mg/kg per day from P11-20)	S.C.	Sprague-Dawley rats (Divided into 2 groups to counterbalance order of Barnes & Morris acquisition tasks).	Straight channel swim (P61) Barnes maze (P62 or P77) Morris water maze acquisition (P62 or P77) Morris water maze cued learning (P70) Morris water maze working memory task (P85)	No effect on swimming speed. No effect on Barnes maze performance – no effect on spatial memory. Impaired Morris water maze acquisition only in group that was tested earlier starting on P62, no effect on group who started on P77 – implies transient reference memory impairment that recovers over time. No effect on cued Morris water maze task. No effect on working memory Morris water maze task.
Moyano et al. (2005)	MDMA (2 x 10 mg/kg for 4 days)	I.P.	Wistar rats	Passive Avoidance Task (retention tested 24 hours later, training started 7 days post drug treatment)	Binge regime produced no effect on performance suggesting no effect on long-term memory consolidation (subsequent acute challenge resulted in sensitisation).
Piper et al. (2005)	MDMA 4 x 5 mg/kg per day, 1 injection per hour, given every 5th day from P35-60).	S.C.	Sprague-Dawley rats	Object Recognition Task (15 & 30 minute delays, tested 4 days after drug treatment finished).	Reduced attention (visited it less & spent less time near it) to novel object with the 30 minute delay.

Author (date)	Drug regime (dose)	Method of Delivery	Subjects	Cognitive Task (s)	Results/Memory Effects
Able et al. (2006)	MDMA (4 x 15 mg/kg in 1 day)	S.C.	Sprague-Dawley rats	Straight channel swim (3 days post drug treatment) Cincinnati water maze (4 days post drug treatment) Morris water maze acquisition (12 days post drug treatment) Morris water maze reversal (19 days post drug treatment) Object recognition (26 days post drug treatment, used 1 hour delay)	No effect on swimming speed. Impaired Cincinnati performance. No effect on Morris water maze acquisition. No effect on Morris water maze reversal. Impaired on Morris water maze probe trials conducted after each phase where platform was removed. No effect on object recognition performance.
Skelton et al. (2006)	MDMA (2 x 20 mg/kg per day from P11 to P20).	S.C.	Sprague-Dawley rats Group 1 (tested P30 - 40) Group 2 (tested P180 – 360)	Straight channel swim Cincinnati water maze Morris water maze acquisition Morris water maze reversal Morris water maze shifted/reduced platform	No effect on swimming speed – no difference between the 2 age groups. Impaired Cincinnati performance (significantly longer latencies, more errors) in younger animals but not in the older group. Impaired performance in Morris acquisition, probe trials, reversal phase & shifted/reduced phase (significantly longer latencies, greater distance travelled, greater cumulative distance) – both age groups impaired.

Author (date)	Drug regime (dose)	Method of Delivery	Subjects	Cognitive Task (s)	Results/Memory Effects
Skelton et al. (2008)	MDMA Group 1- 4 x 15 mg/kg over a single day. Group 2 - 4 x 15 mg/kg over 4 weeks (1 injection per week)	S.C.	Sprague-Dawley rats	Straight channel swim (6 days post drug) Cincinnati water maze (7-12 days post drug) Morris water maze acquisition (14-19 days post drug) Morris water maze reversal (21-26 days post drug) Morris water maze shifted/reduced platform (28-33 days post drug) Novel object recognition (35-39 days post drug) Novel place recognition (40 days post drug)	No effect on swimming time in straight channel. Impaired Cincinnati performance (significantly longer latencies, more errors) – no difference between the two MDMA groups. No effect on Morris acquisition & reversal phase. Both MDMA groups impaired (significantly longer path to reach platform) on Morris shifted & reduced phase. No effect on object recognition. No effect on place recognition.
Skelton et al. (2009)	MDMA (4 x 10 mg/kg per day from P11 to P20).	S.C.	Sprague-Dawley rats	Straight channel swim (9 days post drug) Morris water maze acquisition (10-16 days post drug) Morris water maze reversal 1 (17-23 days post drug) Morris water maze reversal 2 (24-30 days post drug) Cincinnati water maze (31-40 days post drug)	No effect on swimming speed Impaired Morris acquisition, reversal 1, reversal 2 & probe trials (significantly longer latencies, greater path length & cumulative distance travelled). Impaired Cincinnati performance (significantly increased latency & errors).

P - postnatal day I.M. – intramuscular injection S.C. – subcutaneous injection I.P. – intraperitoneal injection

DMTS and DNMTS Tasks

One of the earliest studies to examine both the acute and chronic effects of MDMA exposure on cognitive functioning using a DMTS task was that of LeSage et al. (1993). After completion of the acute phase of the study where pigeons had been trained to perform a DMTS task they were administered repeated injections MDMA. There was no difference in performance before and after chronic MDMA treatment suggesting no lasting deficits in performance. However there was evidence of drug tolerance when acute challenges of MDMA were administered. Therefore LeSage et al. (1993) found that while acute MDMA exposure impaired memory performance chronic MDMA treatment did not produce long-term memory impairments. Using a similar task Marston et al. (1999) found a binge regime of MDMA impaired DNMTP performance during drug administration days and this deficit did not improve 16 days post drug treatment. Marston et al. (1999) argued these delay-dependent impairments suggested binge MDMA exposure produced harmful long-term effects on memory.

Using rhesus monkeys the effects of binge MDMA exposure on cognitive performance was assessed by Taffe et al. (2001). They utilised a DNMTS task, a progressive ratio task and a self-ordered spatial search (SOSS) task. After performance had stabilised, a binge regime of MDMA was administered. During the week of drug treatment performance was significantly impaired on all tasks but these deficits were transient with performance returning to normal the week after drug administration. Taffe et al. (2001) concluded that acute MDMA administration impairs cognitive performance but binge MDMA treatment does not produce long-term deficits.

Using a battery of cognitive tasks Frederick et al. (1995) and Frederick and Paule (1997) examined the effects of acute and chronic regimes of MDMA on Rhesus

monkeys. An operant test battery (OTB) was used that comprised of cognitive tasks including a time estimation task, a DMTS task, a progressive ratio task, a lever pressing sequence task that assessed learning and a conditional discrimination task. Subjects received training on the various cognitive tasks and an acute phase of the experiment was conducted two weeks before the chronic regime began. Performance was significantly affected by acute MDMA exposure but the chronic regime produced no long-term effects. It was also noted that performance on all the cognitive tasks showed evidence of drug tolerance (Frederick et al., 1995). In a later study Frederick et al. (1998) administered a binge regime of MDMA to Rhesus monkeys. OTB performance was negatively affected by acute MDMA administration but there were no long-term effects of binge MDMA treatment. There was also evidence of drug tolerance on OTB performance with subsequent acute MDMA challenges.

In conclusion the evidence for chronic or binge MDMA exposure producing memory deficits in DMTS type tasks is mixed, with the majority of studies failing to produce evidence of impairments. It would definitely appear that acute MDMA exposure disrupts DMTS performance however; it remains unclear as to whether chronic or binge regimes of MDMA produce long lasting impairments in these tasks.

Object Recognition Tasks

Performance in tasks utilising nonspatial stimuli have also shown impairments following binge MDMA administration. For example Morley, Gallate, Hunt, Mallet and McGregor (2001) found binge MDMA treated rats were impaired on an object recognition task using a 15 minute delay but not a 60 minute delay (possibly due to a floor effect where all rats performed poorly in this condition). Therefore Morley et al.

(2001) found evidence of a memory impairment after a high dose binge regime of MDMA.

MDMA research has been criticised for using adult rats whereas MDMA use often begins in adolescence (Piper & Meyer, 2004). Therefore some researchers have administered regimes of MDMA to younger rats to investigate the developmental effects on cognition. Chronic research has also been criticised for not representing the patterns of use reported in human studies that typically involve intermittent use and occurs over longer time periods than that used in animal research (Piper & Meyer, 2004). To try and more closely resemble human MDMA use, Piper and Meyer (2004) administered a chronic regime of MDMA to adolescent rats. When tested in adulthood they were impaired on an object recognition task with a delay of 15 minutes. Therefore, Piper and Meyer (2004) argued that using an intermittent MDMA regime, closer to that found in human MDMA use, impaired non-spatial working memory. In an extension of this study Piper, Fraiman and Meyer (2005) used a different regime of MDMA and found MDMA treated animals showed impaired object recognition performance with a 30 minute delay but not with a 15 minute delay.

Using a short course binge regime of MDMA Able et al. (2006) found no significant impairments on an object recognition task with an hour delay. Skelton et al. (2008) also found MDMA treatments did not affect object or place recognition performance using an hour delay.

In conclusion, despite conflicting findings as to which delay period produces impairment, in object recognition tasks it is clear that chronic and binge MDMA exposure can affect performance in this paradigm. Therefore, chronic and binge MDMA regimes do seem to produce impairments in non-spatial memory.

Maze Tasks

Various maze tasks have also been used to assess the effects of chronic and binge doses of MDMA on cognition. The earliest study to examine the effects of binge MDMA exposure on maze performance was conducted by Slikker et al. (1989) who utilised a complex 24 arm maze to assess memory performance. Even though binge MDMA treatment produced a fifty percent reduction in 5-HT concentration in the frontal cortex and hippocampus of the rats there was no significant effect on maze performance. Therefore, Slikker et al. (1989) found binge MDMA treatment did not impair spatial memory performance.

One of the simplest maze layouts is that of the T-maze which consists of a long runway that splits at the top where the rat can go either left or right. This task assesses spatial memory whereby the rat has to alternate which direction it goes at the top of the maze. Therefore it must remember which direction it went on the previous trial. Ricaurte et al. (1993) used this procedure to assess the effects of a chronic regime of MDMA. While rats showed a significant reduction of brain 5-HT their performance on the cognitive task did not significantly differ from drug free controls. Therefore, Ricaurte et al. (1993) suggested chronic MDMA treatment did not impair memory as assessed by performance in a simple spatial alternation task.

To assess spatial memory one of the most commonly used mazes is the Morris water maze (MWM) (D'Hooge & De Deyn, 2001). This paradigm involves a large circular pool filled with opaque fluid (Morris, 1984). Subjects (typically rats and mice) are placed in the maze at a starting point and are trained to swim to a submerged escape platform that is placed within the maze (D'Hooge & De Deyn, 2001). This platform is not visible to the rats and therefore requires them to use spatial cues to remember its location (Morris, 1984). The standard Morris water maze procedure is

used to assess reference memory as the location of the platform remains the same between trials (Lindner, Balch & VanderMaelen, 1993) and hence involves trial independent long-term stable task rules (Frick, Baxter, Markowska, Olton & Price, 1995). The time taken to reach the platform (latency), the length of the path to reach the platform and cumulative distance from the platform are often used as performance measures (D'Hooge & De Deyn, 2001). After acquisition has occurred probe trials are usually conducted where the platform is removed and the time that the rat spends in the old platform position, referred to as the target site or target quadrant is measured (D'Hooge & De Deyn, 2001). To assess a subject's ability to change their behavior and learn a new location a reversal phase is often conducted where the platform is shifted to a different quadrant within the maze (Morris, 1984). The amount of time the subject spends in the old location and how quickly they learn the new location is usually examined.

The Morris water maze can also assess working memory using a delayed matching to place (DMP) task (Morris, 1984) or matching-to-sample procedure (Vorhees, Reed, Skelton & Williams, 2004). The platform is shifted to a new position each training session and there are multiple trials per session to examine learning (Morris, 1984). This procedure is argued to involve working memory as platform location changes between sessions requiring a more short-term memory process that involves a temporal component where the subject must remember not only the type of stimulus presented but also when the stimulus was presented (Frick et al., 1995).

One of the earliest studies to examine the effects of chronic MDMA on cognition using a working memory Morris water maze task was conducted by Robinson, Castaneda and Whishaw (1993). Initially MDMA treated rats were significantly impaired at finding a shifting platform during the first trials of each session. However they were able to learn the task and there were no deficits on

memory tests conducted 24 hours later. Therefore MDMA treatment significantly impaired the ability to acquire an efficient search strategy when presented with a new spatial problem (Robinson et al., 1993). This impairment was temporary as by the fourth session performance did not differ from controls. Hence binge MDMA exposure did not produce long lasting spatial memory deficits.

Researchers have examined the developmental effects of MDMA by administering chronic regimes of MDMA to adolescent rats and examining their ability to acquire maze tasks in adulthood. Broening, Morford, Inmand-Wood, Fukumura and Vorhees (2001) administered a regime of MDMA to young rats for a period of ten days. When tested in adulthood they were impaired on acquisition, reversal and probe trials in the Morris water maze. However there were no significant impairments in performance on a cued version of the task. Williams et al. (2003) also administered a chronic regime of MDMA to adolescent rats and tested them in adulthood. They showed impairments on all measures of performance (latency, path length and cumulative distance) namely acquisition, reversal and reduced platform (where the size of the platform is made smaller) Morris water maze tasks. Also during acquisition probe trials MDMA treated rats were significantly impaired while performance on a cued version of the task was unaffected. Using a standard Morris water maze task Vorhees et al. (2004) found chronic MDMA treated rats were significantly impaired on task acquisition and probe trials but no impairments in cued maze or working memory Morris water maze performance.

To assess whether memory deficits seen after MDMA exposure during adolescence produce long-term effects Skelton, Williams and Vorhees (2006) administered a chronic regime of MDMA (from P11 - P20) and then assessed cognition in both adolescent rats (P30 – P40) and older rats (P180 to P360). During acquisition, reversal and a reduced platform phase of the Morris water maze both

MDMA treated groups took significantly longer and travelled a greater distance than controls. Both MDMA groups were also significantly impaired during probe trials of the Morris water maze spending less time in the target quadrant suggesting MDMA exposure produced long-term impairments. More recently Skelton et al. (2009) found adolescent rats treated with a chronic regime of MDMA were significantly impaired during acquisition and subsequent probe trials of a Morris water maze task. Reversal phase and shifted/reduced platform performance was also impaired in MDMA treated rats. Therefore chronic MDMA exposure in adolescence produces long lasting impairments in reference memory and cognitive flexibility in adulthood, however cued learning and working memory appear unaffected.

Also using a Morris water maze Sprague, Preston, Leifheit and Woodside (2003) examined the effects of binge MDMA on memory performance. During acquisition there were no significant differences between MDMA treated rats and controls. However, during probe trials the MDMA treated rats showed a significant impairment in the recall of spatial information indicated by worse proximity scores and less time spent in the correct quadrant of the maze during probe trials (Sprague et al., 2003). In addition Able et al. (2006) found a binge treatment of MDMA impaired performance on probe trials but did not significantly impair acquisition of a Morris water maze task. More recently Skelton et al. (2008) compared a short-term binge regime of MDMA with an intermittent regime designed to better model human use. Neither MDMA treatments impaired acquisition, reversal or probe trials on a Morris water maze task. However during a shifted-reduced phase both MDMA groups produced significantly longer paths to reach the platform than controls and there were no differences between the two MDMA treated groups. Therefore there is also evidence that short-term binge courses of MDMA impair reference memory processes.

It should be noted that the Morris water maze has a potential confound as it uses latency (time to find the platform) as a measure of memory performance. However, Mechan et al. (2002) found that chronic MDMA exposure reduced anxiety in rats using a variety of tests. The Morris water maze uses the aversive stimulus of being placed in water to motivate the escape behaviour of rats. Therefore when MDMA is administered it may be that the rats are able to remember where the platform is but they simply do not swim as fast because after MDMA exposure they find the water maze less aversive and are less motivated to escape. Thus the rats produce slower swimming times which are taken as evidence of a memory deficit.

Another common maze used is the Cincinnati water maze (multiple T-maze) that assesses spatial memory and path integration (Skelton et al., 2008). It is comprised of acrylic T-mazes that are filled with water. Rats are placed in the start position and are required to swim to the end of the maze where they can escape. Broening et al. (2001) administered a repeated regime of MDMA to young rats for a period of ten days and found that when tested in adulthood they produced significantly more errors in the Cincinnati maze. Williams et al. (2003) and Skelton et al. (2009) also administered a chronic regime to rats and found MDMA exposure significantly impaired Cincinnati water maze performance.

To assess whether MDMA exposure during adolescence produces long-term effects Skelton et al. (2006) administered a chronic regime of MDMA and then assessed cognition in both adolescent rats and older rats. In the Cincinnati maze the younger rats produced significantly longer latencies and more errors than saline controls. However older rats did not show significant impairments suggesting path integration deficits were temporary.

Utilising a binge regime of MDMA Able et al. (2003) found MDMA treated subjects produced significantly more errors than controls. Skelton et al. (2008) compared the previously used binge MDMA regime from Able et al. (2006) with a new one that was designed to better modeled human use. Both MDMA treated groups produced significantly more errors and significantly longer latencies than saline controls on the Cincinnati water maze but there were no significant differences between the two MDMA groups. In conclusion MDMA exposure appears to impair spatial memory and path integration processes.

The Barnes maze consists of a circular platform that is mounted on a rotatable stand (Vorhees et al., 2004). Around the circumference of the platform are 30 holes. Underneath one of them is a goal box that the rat can enter to avoid aversive stimuli (Vorhees et al., 2004). The goal box is not visible from the surface of the maze and therefore the rats have to learn which hole contains the goal box from extra maze cues. Vorhees et al. (2004) examined the effects of a chronic treatment of MDMA on cognition using a Barnes maze and found no significant differences between MDMA treated rats and controls. However this finding was possibly confounded as performance on this task was poor overall (Vorhees, et al., 2004).

In conclusion there seems ample evidence that chronic and binge MDMA exposure produces memory impairments as assessed using various maze tasks. In particular chronic MDMA treatment seems to produce reference memory impairments in the Morris water maze while leaving working memory and cued learning intact. It would also appear that often these deficits in reference memory are long lasting as the impairments are often present when reversal phases of the maze are utilised.

Other types of cognitive tasks

Not all studies have found evidence of MDMA treatment impairing performance on cognitive or learning tasks. Li, Marek, Vosmer and Seiden (1988) trained rats on a differential-rate-of-low-reinforcement (DRL) 72 seconds task. In this task a response made after 72 seconds produced reinforcement but a response made before 72 seconds had elapsed was not reinforced and the timer was restarted. There was no significant effect of a binge regime of MDMA on performance. When subsequent challenges of acute MDMA were administered performance was impaired. Therefore Li et al. (1988) found evidence that acute MDMA exposure disrupted performance but a binge regime did not produce a noticeable long-term deficits.

In addition Byrne et al. (2000) used a DRL lever pressing acquisition task to assess the effects of chronic MDMA treatment in rats. There was a significant decrease in 5-HT and 5-HIAA levels in several brain regions but no significant differences between MDMA and saline treated rats on measures of task acquisition. Therefore Byrne et al. (2000) found binge MDMA exposure did not impair learning. However in the condition with the longest inter-response delay 25% of the MDMA treated subjects failed to acquire the task suggesting some degree of learning impairment.

Using a repeated acquisition task that involved learning novel lever pressing sequences, Winsauer et al. (2002) examined the effects of binge MDMA exposure on cognition. Squirrel monkeys were assessed on the task before and after two binge regimes of MDMA. No significant differences between baseline and performance after dose 1 and dose 2 of MDMA were found. Therefore Winsauer et al. (2002) found binge MDMA exposure did not affect serial learning.

Finally Moyano, Del Rio and Frechilla (2005) examined the acute and binge effects of MDMA on a passive avoidance task. A week later 5-HT levels were reduced

but no effect on the avoidance task was found. However when later challenged with acute doses of MDMA performance was significantly disrupted indicative of drug sensitisation. Therefore, once again acute MDMA administration appeared to disrupt memory performance but binge exposure produced no discernable long-term effects on memory consolidation (Moyano et al., 2005).

In conclusion there appears ample evidence that acute MDMA administration can disrupt performance on a range of cognitive tasks. However there is also evidence that binge regimes of MDMA produce no long-term effects on an array of learning and cognitive tasks. However, it should be noted that binge MDMA administration does seem to alter behaviour when performance is later challenged with acute doses of MDMA which may be indicative of some underlying impairment.

Conclusion

Although not all studies have found significant impairments in memory function on an array of tasks, there is some evidence to suggest that chronic and binge regimes of MDMA produce specific memory impairments. Seemingly one of the most pronounced effects of chronic and binge MDMA exposure can be seen in maze tasks. Perhaps one of the most interesting findings is that using the Morris water maze both binge and chronic MDMA administration appears to impair reference memory while leaving working memory and cued learning processes intact. However, to date there has not been any research conducted on adolescent or adult rats using the radial arm maze. In fact the only study that seems to have used this apparatus in chronic MDMA research was conducted on rats that were prenatally treated with a chronic regime of MDMA (Thompson et al., 2009). The MDMA treatment produced no effect on maze performance in the offspring of these rats when tested in adulthood. Therefore it was

found that MDMA exposure during pregnancy did not affect the radial arm maze performance of offspring later in life. However, this finding does not answer whether MDMA exposure would affect radial maze performance in rats who are directly administered the drug rather than being exposed via their pregnant mothers.

The partially baited radial arm maze paradigm is a particularly useful paradigm as it enables both reference and working memory processes to be examined simultaneously. By using this paradigm the previous research examining the binge and chronic effects of MDMA on Morris water maze performance can be extended by allowing working and reference memory processes to be investigated using the same procedure. Therefore the second part of the current thesis will administer binge regimes of MDMA to rats to examine the drugs effects on performance in a partially baited radial maze procedure.

General Binge MDMA Method

Apparatus/Materials

The radial arm maze and other apparatus used in the binge section of the thesis was the same as that used in the previous studies of this thesis (see Figure 2 in the General Acute MDMA Method section for diagram of the radial maze and details of other materials used).

Procedure

Pretraining: The procedure for this phase was the same as that used in the acute studies (see General Acute MDMA Method section for details).

Training: The basic training procedure used was the same as that used in the acute studies (see General Acute MDMA Method section for details).

Pharmacological Procedure: The binge studies used a between-subjects experimental design. Rats were randomly divided into an experimental binge group and a saline control group. Experimental rats received a binge regime of MDMA that consisted of 4 injections of 10 mg/kg MDMA that were administered two hourly over the period of one day. The control group received 4 injections of 0.9% saline that were also administered two hourly. After the drugs were administered in the binge studies, rats were then given the training procedure outlined above. Again all drugs were given via i. p. injection.

Statistical Analyses: All inferential statistics were calculated using an alpha level of 0.05. All p-values are given to two decimal places.

Study 3: Binge Effects of MDMA on Radial Arm Maze Acquisition

Previous research that has examined the effects of chronic and binge regimes of MDMA on cognitive processes has produced mixed results (see Chronic MDMA Introduction for a review). More specifically in terms of learning processes the effect of chronic and binge MDMA exposure on the ability to learn or acquire tasks has also resulted in conflicting findings depending on the type of cognitive task utilised. For example the administration of binge regimes of MDMA has failed to produce deficits in acquiring tasks involving learning to respond on DRL schedules (Li et al., 1988), lever pressing (Byrne et al., 2000), lever sequence pressing (Winsauer et al., 2002), and passive avoidance (Moyano et al., 2005).

While MDMA exposure failed to produce learning deficits in the above cognitive tasks there is research that has found the chronic and binge MDMA exposure impairs the learning of various maze tasks. However some of these studies have only produced evidence of transient memory deficits. For example Robinson et al. (1993) found initially MDMA treated rats were significantly impaired at finding the shifting platform in a Morris water maze task during the first couple of trials each session. However by the end of training they were able to learn the task and were performing at a similar level to controls.

The finding that binge MDMA exposure may produce transient effects has also been found in physiological studies. For example Scanzello, Hatzidimitriou, Martello, Katz and Ricaurte (1993) examined the effect of a chronic regime of MDMA (4 x 10 mg/kg) on serotonergic neurotoxicity in rats. Two weeks after drug exposure there were significant reductions in 5-HT markers. However after sixteen weeks there was

evidence of recovery in some brain regions and by thirty weeks there was almost full recovery. Even when using larger doses of MDMA there has been evidence of recovery. For example Sabol, Lew, Richards, Vosmer and Seiden (1996) administered a binge regime of MDMA to rats (8 x 20 mg/kg) and then measured brain 5-HT concentration. A significant reduction in 5-HT levels was found two weeks after drug exposure but by sixteen weeks there was some initial evidence of recovery that increased up to 52 weeks later. In addition there has been evidence of behavioural recovery of function occurring twelve weeks after MDMA exposure in tasks assessing locomotor activity (Brennan & Schenk, 2006). Therefore one explanation for the apparent transient behavioural effects seen in some of the studies that have examined the binge effects of MDMA on cognition may be the result of physiological recovery

There has also been some evidence that chronic MDMA exposure has produced more long-term cognitive deficits which remain several weeks after drug exposure. For example Broening et al. (2001) found MDMA treated rats were significantly impaired on a Morris water maze and a Cincinnati maze task and these impairments were still evident more than 50 days post drug treatment. Similarly chronic and binge MDMA exposure produced long lasting impairments in sequential or path integration and reference memory as assessed using the Cincinnati and Morris water mazes (Williams et al., 2003; Able et al., 2006; Skelton et al., 2008; Skelton et al., 2009). Whereas Sprague et al. (2003) found MDMA-treated rats were significantly impaired in their ability to recall spatial information during Morris water probe trials. Vorhees et al. (2004) found several reference memory measures of Morris water maze performance were significantly impaired more than forty days after MDMA administration indicating a long-term cognitive impairment. Finally Skelton et al. (2006) found MDMA treated rats showed reference memory impairments in the Morris water maze

when tested 160 and even 340 days after drug treatment indicating a notable long-term cognitive deficit.

Therefore chronic and binge MDMA exposure can produce deficits in the ability to acquire various cognitive tasks. Specifically it impairs maze performance in particular reference memory and path integration processes seem more susceptible to the effects of MDMA administration. However cued learning and working memory tasks appear to be unaffected. Therefore one possible explanation for the conflicting task acquisition findings is that chronic and binge MDMA exposure is more likely to impair tasks that have a high reference memory component to them. Thus the finding that MDMA exposure has not produced impairments in the acquisition of some operant based tasks could be that these tasks do not involve reference memory processes to the same extent as Morris and Cincinnati water maze procedures.

However, it should be noted that in many of these maze studies the order in which the tasks are conducted may produce a potential confound. The majority of the studies that have examined the effects of MDMA exposure on maze performance have started testing with the standard Morris water maze and Cincinnati maze tasks and generally test cued learning and working memory later. As there is evidence that the effects of binge MDMA regimes may produce transient effects it is possible that the reason cued learning and working memory processes seem unaffected by MDMA exposure may be that by the time these tasks are conducted subjects may have began to experience recovery of function from the effects of the drug regime.

Also when using the Morris water maze to test for reference and working memory it requires different procedures that need to conducted during different times as this apparatus does not allow for the simultaneous assessment of working and reference memory processes. Therefore this introduces potential confounds in trying to

ascertain which cognitive processes are affected by chronic and binge regimes of MDMA. Hence the partially baited radial arm maze may provide an invaluable opportunity to examine the effects of MDMA exposure on both working and reference memory processes concurrently.

Drug Tolerance or Sensitisation

Drug tolerance occurs when a subject becomes progressively less responsive to a drug and requires more of the drug to have the original effect (Parrott, 2005). Ecstasy users have reported having to increase the amount of the drug they take to experience the positive effects of the drug (Parrott, 2001). For example while first time users generally take one tablet of Ecstasy, regular users often take two to three tablets and there are reports of long-term users taking up to 25 tablets (Parrott, 2005). These findings indicate Ecstasy users become tolerant to the effects of the drug. Tolerance is an important component in DSM-IV criteria for drug dependence and abuse (Cottler et al., 2001) and therefore examining whether repeated MDMA exposure produces drug tolerance is worthy of further research. However, animal literature that has examined the effects of MDMA exposure has mixed findings with some studies reporting repeated administration of the drug results in drug tolerance while others report drug sensitisation occurs. In contrast to drug tolerance, drug sensitisation occurs when there is a progressive increase in the responsiveness to a drug with repeated administration of the original dose (Ramos, Goni-Allo & Aguirre, 2005). While there are numerous underlying neurochemical causes as to why drug tolerance and drug sensitisation occur, the focus of this thesis is on the behavioural effects of MDMA. Therefore we will investigate whether behavioural drug tolerance or sensitisation occurs following repeated MDMA exposure.

Within animal research there is evidence of behavioural tolerance whereby animals that have been previously exposed to MDMA are less affected when re-exposed to the drug compared to subjects that did not experience pre-exposure (Shankaran & Gudelsky, 1999; Piper, Vu, Safain, Oliver & Meyer, 2006; Brennan & Schenk, 2006). Evidence of behavioural tolerance resulting from repeated exposure to MDMA has also been found in more complex tasks that assess cognitive functioning (LeSage et al., 1993; Marston et al., 1999). Other studies have also produced evidence of behavioural tolerance developing to MDMA such as Frederick et al. (1995), Frederick & Paule (1997) and Frederick et al. (1998). In these studies repeated exposure to MDMA resulted in a lessening of the initial impairments seen on a large battery of cognitive tasks indicating that performance was less affected as drug administration continued.

In contrast there have also been reports of repeated MDMA exposure producing behavioural sensitisation within the animal literature (Spanos & Yamamoto, 1989; Kalivas, Duffy & White, 1998; Modi, Yang, Swann & Dafny, 2006). In addition there is evidence that behavioural sensitisation can occur after repeated MDMA administration in tasks that investigate cognitive functioning (Li et al., 1989; Moyano et al., 2005).

Therefore in the final phase of the current study, to try and clarify whether MDMA exposure produces behavioural tolerance or sensitisation, both the binge MDMA treated and saline control rats were subsequently administered acute doses of MDMA (4.0 mg/kg) to examine what effect this would have on their performance once they had acquired the task. Specifically, this study investigated whether the binge treated rats would show evidence of tolerance which would be present if their performance in the maze task was less impaired than those of the saline controls treated with acute MDMA. Alternatively evidence of sensitivity to the acute effects of

MDMA would be indicated by greater impairment in the binge treated rats compared to the saline controls after acute MDMA administration.

The reasons why such conflicting findings as to whether repeated MDMA exposure results in tolerance or sensitisation are unclear. However Brennan and Schenk (2006) offer one possible explanation that involves the regime of MDMA the subjects are exposed to. It has been suggested that repeatedly administering low doses of MDMA may result in sensitisation developing to the effects of MDMA while tolerance may develop following the administration of large chronic or binge doses (Brennan & Schenk, 2006).

The Current Investigation

Previous studies concerning MDMA's effects on learning using animal subjects have produced mixed results. Previous research has produced evidence that acute and chronic or binge MDMA exposure may affect reference memory more than working memory (Harper et al., 2005; Harper et al., 2006; Kay et al, 2009; Broening et al., 2001; Williams et al., 2003; Sprague et al., 2003; Vorhees, 2004; Skelton et al., 2006; Able et al., 2006; Skelton et al., 2008; Skelton et al., 2009). However, to date no study has used the partially baited radial arm maze paradigm to study the effect of binge MDMA exposure performance of adult rats acquiring this procedure. This paradigm is advantageous in that it can differentiate between working memory and reference memory impairments. Therefore, the partially baited radial arm maze task enables the assessment of an issue not specifically examined in studies utilising tasks that focus only on working memory or are unable to assess working and reference memory simultaneously using the same procedure.

The first part of the current study investigated the effects of a previously used binge (4 x 10 mg/kg) MDMA treatment (Scanzello et al., 1993; Brennan & Schenk, 2006) on the acquisition of the partially baited radial arm maze paradigm. Rats were administered a binge regime of MDMA and their ability to learn or acquire the radial arm maze task was compared against control rats that received saline. Therefore the current study hypothesises that the MDMA treated rats would show impaired learning compared to saline controls and specifically this impairment would be the result of a reference memory deficit.

The second part of the current study investigated the effects of re-administering the binge regime of MDMA to the rats. This phase was conducted due to research (Robinson et al., 1993) that suggests MDMA may produce transient effects on memory. Due to a period of approximately eight to ten weeks between the initial drug administration and the commencement of radial maze training in the initial phase of the study, there is the possibility that any transient cognitive deficits would not be evident. Therefore the rats in the experimental MDMA group were re-treated with the same regime of MDMA used in the first phase to examine whether further exposure would produce a deficit in performance in the radial arm maze task after acquisition had occurred. It was hypothesised that further MDMA exposure would result in a deficit in performance. Specifically it is hypothesised that the MDMA treated rats will be less accurate than the saline controls and that they will produce more reference memory errors than working memory errors.

Finally the last part of the current study examined whether repeated MDMA exposure would produce evidence of drug tolerance or sensitisation. After the first two phases of the study had been completed all rats were challenged with an acute dose of MDMA to examine what effect this would have on rats that had already learnt the task and had already been exposed to repeated binge regimes of MDMA. As previous

research (Brennan & Schenk, 2006) suggests pre-treatment with chronic/binge regimes of MDMA may produce tolerance to the effects of MDMA, it was hypothesised that repeated MDMA exposure would result in drug tolerance. This behavioural tolerance would be revealed by the rats that were previously treated with a binge regime of MDMA would be less impaired than the control rats when the subsequent acute MDMA treatments were administered.

Method

Subjects

The subjects were twenty white male Sprague-Dawley rats that were approximately five months old at the beginning of the current study. The rats were housed individually. To reduce the number of animals used within the laboratory the current rats were not experimentally naïve as they had previously participated in a study examining the effects of binge MDMA on anxiety that used an emergence test. However they had never received training in a maze or operant procedure previous to the current investigation.

The rats were kept at 85-90% (between 237 and 382 grams) of their free feeding body weight and began training in the current study five days after reaching this weight. They had continuous access to water and were kept on a 12:12-hour dark:light cycle and were run during the light phase of this cycle.

Apparatus/Materials

The maze and reinforcers used were the same as those stated in the general method section. Drugs used were saline 0.9 % and MDMA 10 mg/kg, which were

prepared on the day of use by dissolving to the required dose in 0.9 % saline solution. During the tolerance phase of the experiment MDMA 4.0 mg/kg was used and it was also dissolved to the required dose in 0.9 % saline solution.

Procedure

The study used a between-subjects experimental design where one group of rats received binge doses of MDMA and the other received saline. Rats were assigned to the experimental and control group (10 rats in each group). Rats in the experimental binge group received injections of 10 mg/kg of MDMA while control rats were given 0.9 % saline. Each rat was given four injections at two hour intervals. Unfortunately one of the rats in the experimental group died after receiving only a few sessions of training and so this rat's data are excluded from all analyses. Therefore, there were only nine rats in the binge group, compared to ten in the saline control group.

Each rat was assigned a set of four reinforced arms and four non-reinforced arms within the maze. The binge and control rats had the same sets of reinforced and non-reinforced arms, so that the first rat in both groups had the same reinforced arms so that there were matched pairs of rats. This was done to control for difficulty of the task in case some sets of maze arms were easier to learn that others. Training was conducted in the same way as outlined in the General Acute MDMA Method section with the habituation sessions commencing approximately eight weeks after being given either the binge regime of MDMA or saline control.

Acquisition: During the acquisition phase each rat was only given one session (consisting of three trials) in a day. Rats were run in numerical order within their experimental groups, starting with rat one and finishing with rat ten. Each group of rats were given five sessions of training per week. In the first phase of the experiment rats

had to reach a criterion of a group average of at least 85% accuracy for five consecutive days before they had been considered to have achieved acquisition. This took twenty three training sessions after which time the second phase of the experiment began.

Re-dosing: This phase examined what effect additional exposure to binge doses of MDMA would have on performance in a task that had already been acquired. Therefore during the second phase the injections were repeated where the experimental group again received MDMA (4 x 10mg/kg) and the control group were administered saline (0.9%). As in the previous phase, each rat received four injections in total with a two hour period between injections. Unfortunately two of the rats that received the binge doses of MDMA died during this procedure leaving seven rats in the experimental group and ten in the control group. No rats were run during the day of injections and they were also given an additional rest day after this. Maze running commenced after this rest day and rats were run using the same procedure as in the previous phase of this study. This phase continued for another twenty three sessions as by this time performance had stabilised.

Acute Effects: Finally in the third and last phase of the experiment we conducted a study to examine the effects of acute administration of MDMA on both the experimental and control groups. This phase began immediately following the second phase of the study. All drugs were administered via an intraperitoneal injection twenty minutes before running. Rats were run in batches where the first four rats were injected and then twenty minutes after the first rat was injected all four rats were run. Once this batch had completed running the maze the second batch of three was run until all the rats were finished. This study began with all rats receiving an injection of 0.9 % saline and they were then run in the maze as usual. This was followed by three sessions of running without any drugs being administered. Finally on the last day of

the study all rats received an injection of 4.0 mg/kg of MDMA and were then run in the maze as usual.

Results

Acquisition and Re-dosing

In all figures error bars show standard error of the mean. Daily percent correct figures were calculated by averaging across the three daily trials to obtain an average level of performance for the session for an individual. Group means were then calculated for both the experimental binge group and the saline control group. This was done for each daily session for both initial acquisition and the re-dosing phase and these data are shown in Figure 11.

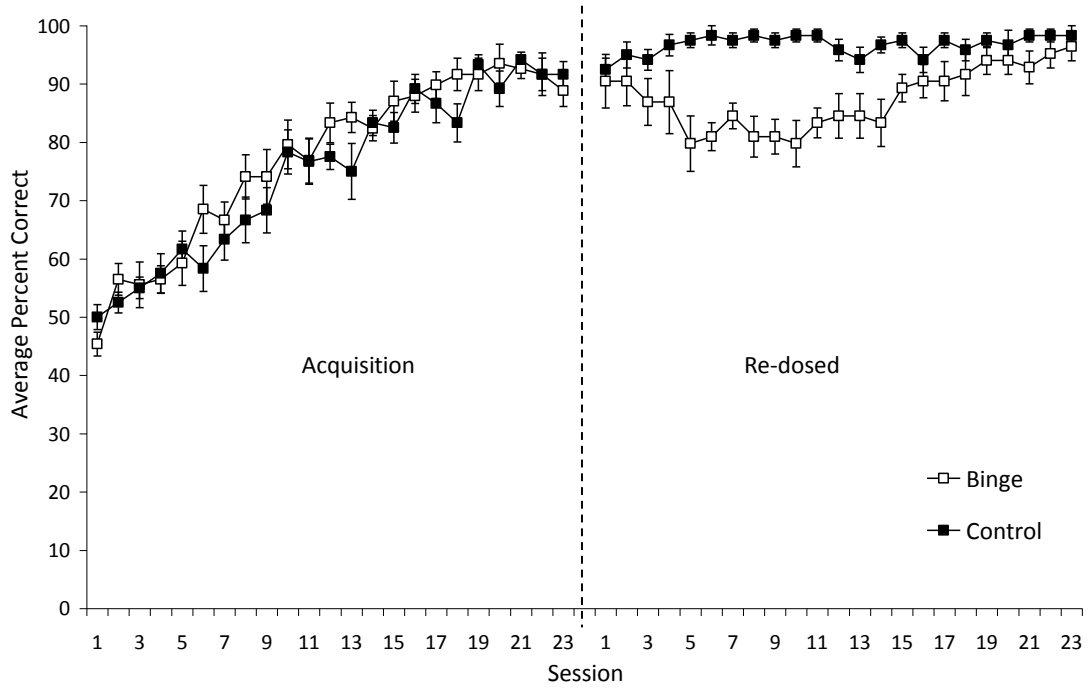

Figure 11: Average percent correct across all rats in the binge and control groups for each session of acquisition training and then for sessions after re-dosing.

Figure 11 shows that during the acquisition phase accuracy increased for both groups across training sessions and there was no visible difference between the binge and control groups in the accuracy and speed in which they learnt the radial maze task. This was confirmed by a two-way mixed ANOVA that found no interaction, $F(22, 374) = 1.07$, $p > 0.05$ ($p = 0.38$) and no main effect for group, $F(1, 17) = 1.24$, $p > 0.05$ ($p = 0.28$). However there was a main effect for training session, $F(22, 374) = 50.02$, $p < 0.05$ ($p = 0.00$).

In addition during the re-dosing phase there was no real difference in accuracy between the groups in the first two sessions. However, by the third session the binge group's accuracy started to decline in comparison to the control group and this trend continued until around session fifteen where performance started to improve until it reached an equivalent level to the control group. Therefore, the experimental binge group did appear to experience some degree of transient impairment during the re-dosing phase. These effects were confirmed by a two-way mixed ANOVA where during the re-dosing phase there was a main effect for session, $F(22, 330) = 3.02$, $p < 0.05$ ($p = 0.00$) and for group, $F(1, 15) = 28.41$, $p < 0.05$ ($p = 0.00$), but these main effects were moderated by a significant interaction between training session and group, $F(22, 330) = 3.54$, $p < 0.05$ ($p = 0.00$).

Average trial completion times (in seconds) for each daily session were calculated for each rat and this data was then used to obtain group averages for both the binge and controls groups. These data are depicted in Figure 12 and show trial completion time decreased for both groups as training continued producing a main effect for session, $F(22, 374) = 20.15$, $p < 0.05$ ($p = 0.00$). However during initial acquisition there was no noticeable difference between the MDMA treated group and the saline control group with no interaction between training sessions and drug

treatment group, F (22, 374) = 1.15, $p > 0.05$ ($p = 0.29$) and no main effect for group, F (1, 17) = 0.14, $p > 0.05$ ($p = 0.71$).

During the re-dosing phase there is an increase in trial completion times for the MDMA treated group that lasted approximately eight sessions. Hence MDMA treated rats took longer than the controls on average to complete their trials during this period which could be indicative of a learning impairment. A two-way mixed ANOVA confirmed these effects with main effects for session, F (22, 330) = 5.76, $p < 0.05$ ($p = 0.00$) and group, F (1, 15) = 12.47, $p < 0.05$ ($p = 0.00$) that were qualified by a significant interaction between session and group, F (22, 330) = 3.73, $p < 0.05$ ($p = 0.00$).

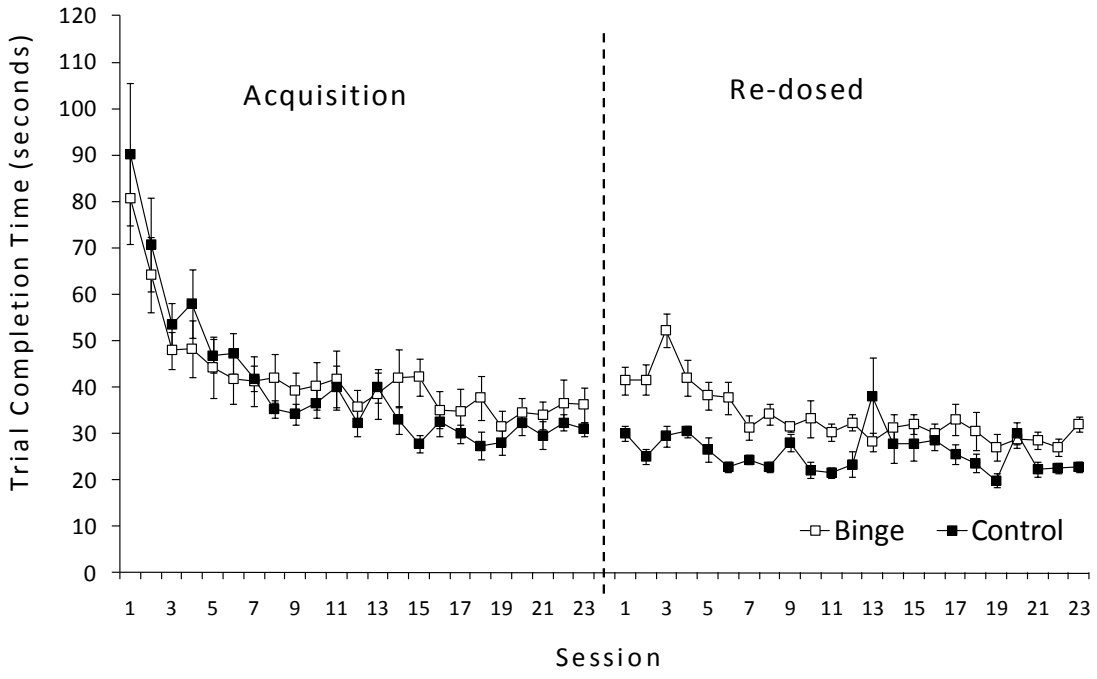

Figure 12: Average trial completion times in seconds across all rats in the binge and control groups for each session of acquisition training and then for sessions after re-dosing.

Working Vs. Reference Memory Data

To examine the difference between types of error, the average number of working memory errors and reference memory errors made per session for each rat was obtained by averaging across the three trials. These figures were then converted into percentage error values in the same way as in the previous studies. Average error percentage values were calculated for both the experimental group and the control group by averaging across the rats in these groups. This was done for each daily session for both the acquisition and re-dosing phases of the study and each error type.

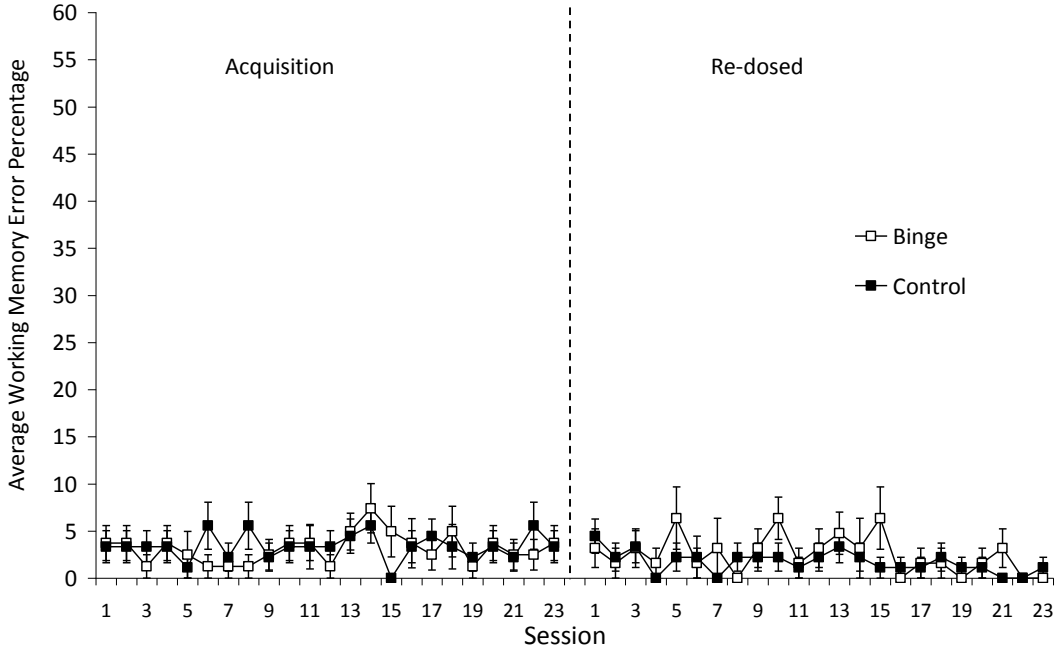

Figure 13: Average working memory error percentages across all rats in both the binge MDMA and saline control groups during acquisition and re-dosing.

For the acquisition data a 2-way repeated measures ANOVA comparing error type and training sessions revealed a significant interaction between error type and session, $F(22, 396) = 36.75$, $p < 0.05$ ($p = 0.00$). Thus there was a significant

difference in the type of errors made over the training sessions. During the re-dosing phase there was no significant interaction between error type and session, $F(22, 352) = 0.93$, $p > 0.05$ ($p = 0.56$) indicating that there was no difference in error type during re-dosing. These effects were examined further by individually analysing the data for working and reference memory errors across training sessions and between groups.

The data for average working memory errors are presented in Figure 13 and show that both groups made few working memory errors during acquisition and there appears to be no clear difference in performance between the two groups. However, the size of the error bars indicates there was some variability in the data. A two-way mixed ANOVA found no significant differences in performance with no significant interaction between group and session, $F(22, 374) = 0.68$, $p > 0.05$ ($p = 0.86$) and no main effects for session, $F(22, 374) = 0.73$, $p > 0.05$ ($p = 0.80$) or group, $F(1, 17) = 0.09$, $p > 0.05$ ($p = 0.77$). Therefore MDMA treatment had no significant effect on working memory errors when acquiring the partially baited radial maze task.

Figure 13 also shows that during the re-dosing phase there were very few working memory errors made and there was no obvious change in the number of working memory errors made across training sessions with no main effect for training session, $F(22, 330) = 1.38$, $p > 0.05$ ($p = 0.12$). There was also no noticeable difference in performance between the MDMA treated and saline control group with no significant interaction between group and training session, $F(22, 330) = 0.79$, $p > 0.05$ ($p = 0.74$) and no main effects for group, $F(1, 15) = 1.37$, $p > 0.05$ ($p = 0.26$). Therefore exposing the rats to another regime of MDMA still had no significant effect on working memory performance.

The data for average reference memory errors are presented in Figure 14 and show that during the acquisition phase initially both groups of rats produced quite a high number of reference memory errors. However, as training progressed and rats

began to acquire the task the number of reference memory errors decreased to a very low level producing a main effect for session, $F(22, 374) = 56.66$, $p < 0.05$ ($p = 0.00$). There were no obvious differences between the MDMA and saline treated groups in Figure 14 and there was no interaction between group and training session, $F(22, 374) = 1.13$, $p > 0.05$ ($p = 0.31$) and no main effect for group, $F(1, 17) = 1.28$, $p > 0.05$ ($p = 0.27$). Therefore binge MDMA exposure produced no significant reference memory impairment compared to saline treatment during the training phase.

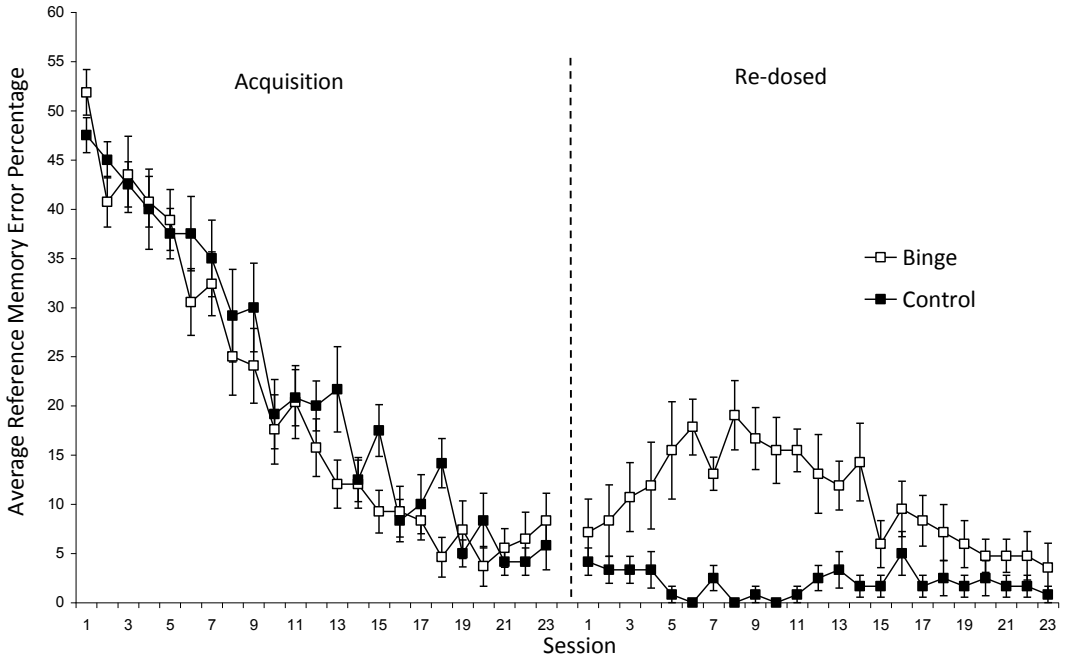

Figure 14: Average reference memory error percentages across all rats in both the binge MDMA and saline control groups during acquisition and re-dosing.

Throughout the re-dosing phase of the experiment the number of reference memory errors made by the control group still continued to decrease slightly levelling out at a very low number of errors. However, the MDMA group during the same period began making more reference memory errors and this continued to increase for approximately eight sessions when it began to gradually decrease until it levelled out

to reach a similar level of performance to the control group. There were main effects for session, $F (22, 330) = 2.67$, $p < 0.05$ ($p = 0.00$) and group $F (1, 15) = 36.53$, $p < 0.05$ ($p = 0.00$), however these main effects were qualified by a significant interaction between group and session, $F (22, 330) = 4.14$, $p < 0.05$ ($p = 0.00$). Therefore re-exposing the rats to MDMA during the re-dosing phase had a disruptive effect on reference memory processes across this phase of the study.

Acute Drug Effects

Percent correct figures were calculated by averaging across the three daily trials to obtain an average level of performance for the session for an individual. Group averages for both the experimental and control rats were calculated and these data are depicted in Figure 15. This figure shows that both groups produced a high level of accuracy, as shown by average percent correct values exceeding ninety percent, during the acute saline treatment. There was also no obvious difference between groups in their level of performance during saline treatment.

During the acute MDMA session both groups produced lower levels of accuracy than those seen during the saline session. Interestingly the binge MDMA group produced a higher average percent correct value than the saline control group during this session. This suggested that the saline controls were more affected by the acute dose of MDMA than the experimental group. These effects were confirmed by a two-way mixed ANOVA that revealed a significant interaction between acute drug treatment and group, $F (1, 15) = 9.58$, $p < 0.05$ ($p = 0.00$). There were also main effects for acute drug treatment, $F (1, 15) = 192.18$, $p < 0.05$ ($p = 0.00$) and group, $F (1, 15) = 6.01$, $p < 0.05$ ($p = 0.03$).

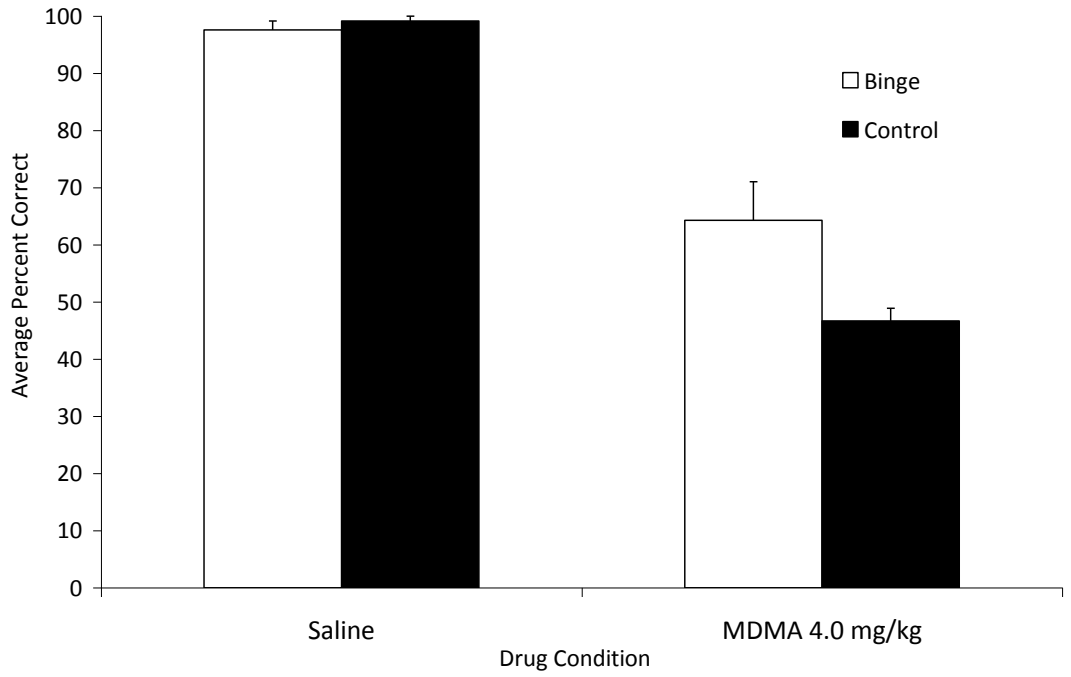

Figure 15: Average percent correct across all rats for both the experimental binge group and the saline control group during the acute phase.

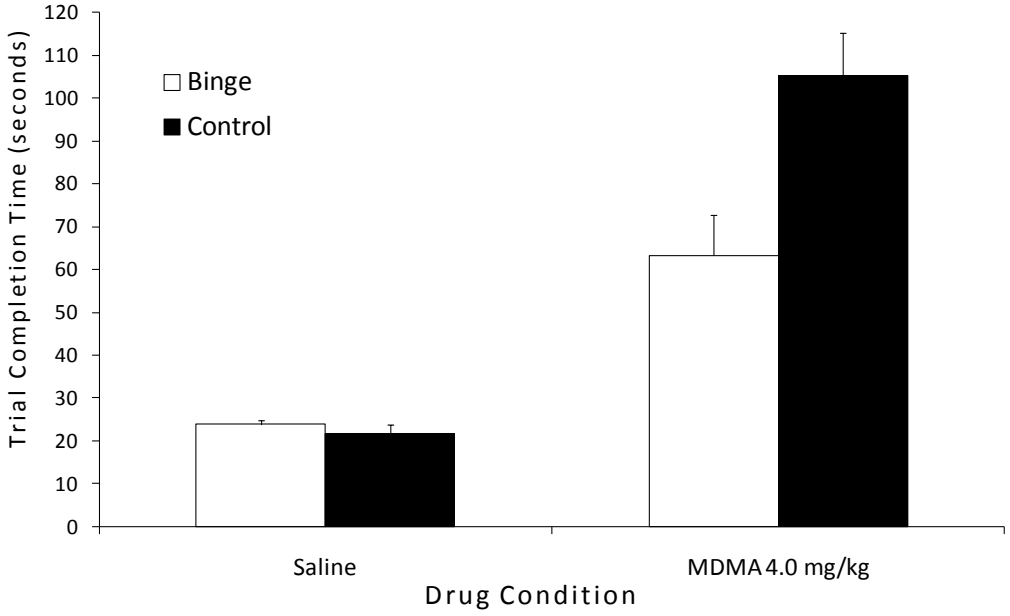

Figure 16: Average trial completion time in seconds across all rats for both the experimental binge group and the control group during the acute tolerance study.

Average session trial completion times (in seconds) were calculated for each individual rat. These data are depicted in Figure 16 and show both the binge MDMA and control groups produced similar low trial completion times during saline administration. However, during the MDMA session both groups produced much longer trial completion times compared to the saline session. Also the control group produced much longer trial completion times than that of the binge MDMA group. Again this suggested that saline controls were more affected by the acute dose of MDMA than the binge MDMA group. These effects were confirmed with a significant interaction between acute drug treatment and group, $F(1, 15) = 8.91$, $p < 0.05$ ($p = 0.01$). There was also a main effect for acute drug treatment, $F(1, 15) = 69.32$, $p < 0.05$ ($p = 0.00$) and a main effect for group, $F(1, 15) = 7.54$, $p < 0.05$ ($p = 0.02$).

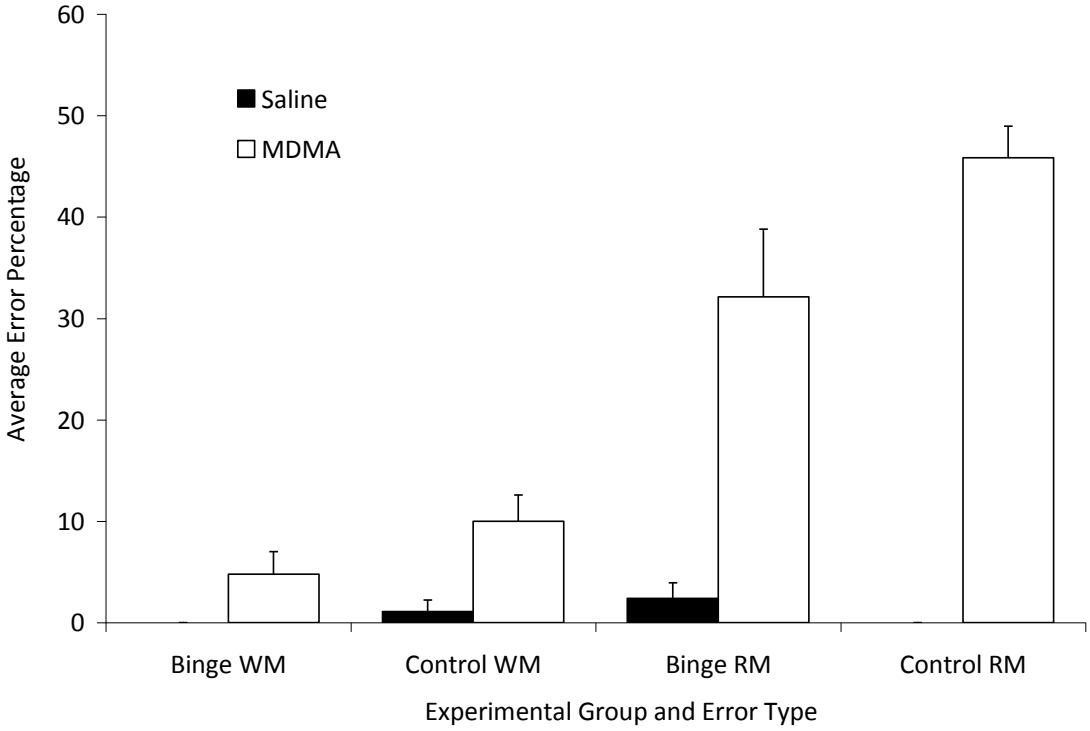

Figure 17: Average error percentage across all rats in both the binge MDMA and saline control groups during the acute phase where all rats were administered 0.9 % of saline and 4.0 mg/kg of MDMA. RM stands for reference memory and WM stands for working memory.

The number of working and reference memory errors made per session for each rat were calculated and converted into percentage error values. Average error percentage values were calculated for both the binge MDMA group and the saline control group. These data are depicted in Figure 17 and show that saline administration produced very few working memory errors in either group. With acute MDMA exposure the number of working memory errors increased for both groups with the control group producing slightly more working memory errors than the binge MDMA group. However there was no significant interaction between acute drug treatment and group, $F(1, 15) = 1.17$, $p > 0.05$ ($p = 0.30$).and no main effect for group, $F(1, 15) = 2.61$, $p > 0.05$ ($p = 0.13$). A main effect for drug treatment was found, $F(1, 15) = 12.75$, $p < 0.05$ ($p = 0.00$). From Figure 17 it is clear that this difference is due to acute MDMA treatment producing more working memory errors than acute saline treatment.

Figure 17 also shows that very few reference memory errors were made by either group during the saline session. However during the acute MDMA session there were a large number of reference memory errors made by both groups but the saline control group appeared to make more reference memory errors than the binge MDMA group. A two-way mixed ANOVA confirmed this effect with a significant interaction between acute drug treatment and group, $F(1, 15) = 6.01$, $p < 0.05$ ($p = 0.03$). There was a main effect for acute drug treatment, $F(1, 15) = 133.02$, $p < 0.05$ ($p = 0.00$), but no main effect for group, $F(1, 15) = 2.62$, $p > 0.05$ ($p = 0.13$).

To compare the types of error made a 3-way mixed ANOVA was conducted. There was no 3-way interaction between memory type, acute drug treatment and group, $F(1, 15) = 1.91$, $p > 0.05$ ($p = 0.19$). Therefore there was no significant difference between the types of errors (working and reference) made during drug treatment (acute MDMA and acute saline) between the binge MDMA group and the

saline controls. There was also no interaction found between memory type and group, $F(1, 15) = 0.30$, $p > 0.05$ ($p = 0.60$). Therefore there was no significant difference between the number of working and reference memory errors made between the binge MDMA and control groups. However there was a significant interaction between memory type and acute drug treatment, $F(1, 15) = 51.37$, $p < 0.05$ ($p = 0.00$); indicating that there were significantly more reference memory errors made than working memory errors during the acute MDMA treatment. There was also a significant interaction between acute drug treatment and group, $F(1, 15) = 10.08$, $p < 0.05$ ($p = 0.01$); suggesting that there was a significant difference in the effects of the acute MDMA treatment and acute saline treatment on the two groups where the saline controls were more affected by the acute drug treatment. There were main effects for memory type, $F(1, 15) = 49.84$, $p < 0.05$ ($p = 0.00$), acute drug treatment, $F(1, 15) = 196.89$, $p < 0.05$ ($p = 0.00$) and group, $F(1, 15) = 6.95$, $p < 0.05$ ($p = 0.02$).

Therefore, it would appear that repeated MDMA exposure produces drug tolerance as those in binge MDMA group who have already received MDMA treatment were not as affected as the saline control group who had not been previously exposed to MDMA. Also as in the first half of the thesis it was found that acute MDMA administration produced a decrease in accuracy, an increase in the average amount of time taken to complete a trial. Also of note acute MDMA administration resulted in more reference memory errors than working memory errors, once again indicating that MDMA exposure impairs reference memory processes.

Discussion

To recap, the intention of the current study was to examine whether a binge regime of MDMA would impair the ability of rats to acquire the partially baited radial arm maze task. The administration of a binge regime of MDMA produced no significant differences in terms of accuracy and trial completion time compared to saline controls during the initial acquisition phase. Therefore binge MDMA exposure did not seem to impair learning thus failing to support the hypothesis that the regime of MDMA used would disrupt maze acquisition. Of interest rats produced more reference memory errors than working memory errors during the initial acquisition phase of the task. However, there were no significant differences between the number and type of errors made between the two groups suggesting MDMA exposure did not affect error production.

The current study also investigated whether additional exposure to a binge dose of MDMA would impair performance once the task was learnt. During the re-dosing phase the binge MDMA treated group showed a significant decrease in accuracy and an increase in trial completion time compared to the saline control group. Also during this phase the number of working memory errors made was unaffected, however the rats in the binge MDMA group produced a significant increase in the number of reference memory errors made compared to the saline group. Therefore, during this re-dosing phase we did find evidence of MDMA exposure producing a cognitive deficit supporting the hypothesis that an additional regime of MDMA would impair performance and specifically that this impairment would affect reference memory processes.

Finally we examined whether there would be any difference between the binge MDMA treated group and the saline control group when exposed to acute doses of

MDMA. During the acute phase of the task when rats received saline there were no significant differences found between the binge MDMA treated and control group in accuracy, trial completion time or type of error made. However, when rats received the acute challenges of MDMA performance was significantly affected in both groups. Of note the saline control group showed a greater degree of impairment than the binge MDMA group in terms of decreased accuracy and increased trial completion time. Both groups produced more reference memory errors than working memory errors when administered acute injections of MDMA, however the saline control rats produced significantly more reference memory errors than the binge MDMA group. This supported the hypothesis that behavioural tolerance would occur with repeated MDMA exposure as the rats that had been previously exposed to a binge dose of MDMA were less affected by the acute challenges than the saline controls who had not experienced previous MDMA exposure.

The finding that the initial binge regime of MDMA did not produce learning deficits is in agreement with previous research that has utilised various operant based tasks and found MDMA exposure did not disrupt acquisition (Li et al., 1988; Byrne et al., 2000; Winsauer et al., 2002; Moyano et al., 2005). However this current finding does conflict with research that has utilised more similar maze type procedures to the current study and have found that binge MDMA regimes do produce learning deficits (Robinson et al., 1993; Broening et al., 2001; Willaims et al., 2003; Sprague et al., 2003; Vorhees et al., 2004; Skelton et al., 2006; Able et al., 2006; Skelton et al., 2008; Skelton et al., 2009).

The fact that an additional regime of MDMA was able to produce a transient but significant disruption in performance does concur with previously cited maze studies that have found MDMA exposure disrupts performance. More specifically the current studies finding that this impairment seems to be a reference memory

impairment concurs with previous research that has used the Morris water maze and Cincinnati maze procedures (Robinson et al., 1993; Broening et al., 2001; Willaims et al., 2003; Sprague et al., 2003; Vorhees et al., 2004; Skelton et al., 2006; Able et al., 2006; Skelton et al., 2008; Skelton et al., 2009).

Therefore the current research adds to the previous MDMA literature as it was able to show that a binge MDMA regime impaired reference memory processes while leaving working memory processes intact in a paradigm that was able to simultaneously assess the two processes. However it could be argued that as the deficit in learning was produced after acquisition had taken place, it may be dissimilar in nature to these previous studies that found deficits during task acquisition which suggests MDMA disrupted learning processes.

There are two main possible reasons for the current study's findings that an initial regime of MDMA did not result in impairment while administering an additional regime did produce evidence of albeit a transient but significant deficit in performance. The first explanation is that the initial regime of MDMA was simply not sufficient to produce an effect on cognitive performance. Previous doses of chronic and binge MDMA used have often involved higher doses and also administered them over a period of several days. For example Robinson et al. (1993) administered twice as many of injections of 10mg/kg of MDMA than the current study while Able et al. (2006) and Skelton et al. (2008) used four injections of 15mg/kg in a day. In addition Williams et al. (2003) and Skelton et al. (2006) administered their regime of MDMA (2 x 20mg/kg per day) for a period of ten days. Therefore there is the possibility that the regime used in the current study (4 x 10 mg/kg for 1 day) was insufficient to produce cognitive impairment.

The second possibly more plausible explanation for these finding could be due to the eight to ten week gap between MDMA exposure and the beginning of training in the radial arm maze. Previous research (Robinson et al., 1993; Brennan & Schenk, 2006) has shown that while MDMA may produce behavioural effects they may only be temporary. In addition, research examining the neurochemical effects of MDMA has shown evidence of recovery after MDMA exposure (Scanzello et al., 1993; Sabol et al., 1996). Therefore it is possible that we did not find an initial deficit in learning the radial maze task as during the time period between drug administration and training the rats experienced some recovery of function from the effects of the drug regime used.

The finding that when challenged with an acute dose of MDMA the rats that had previously been administered a large binge dose of MDMA were less impaired than saline controls concurs with several studies that have also found evidence of behavioural tolerance developing with repeated MDMA exposure (Shankaran & Gudelsky, 1999; Piper et al., 2006; Brennan & Schenk, 2006; LeSage et al., 1993; Marston et al., 1999; Frederick et al., 1995; Frederick & Paule, 1997; Frederick et al., 1998). However this finding conflicts with several studies that have found evidence of behavioural sensitisation occurring after repeated MDMA exposure (Spanos & Yamamoto, 1998; Kalivas et al., 2006; Modi et al., 2006; Li et al., 1989; Moyano et al., 2005).

Brennan and Schenk (2006) offer the explanation that this may be due to the type of MDMA regime used where repeated smaller acute doses may result in sensitisation whereas larger chronic or binge regimes may result in behavioural tolerance. Due to the size of the regime used in the current study which previous studies have found it produces significant effects on 5-HT levels to justify its use in binge MDMA studies (Scanzello et al., 1993). Therefore the regime used in the current

study may explain this finding as the dosage used may have been sufficiently large and brief enough to produce behavioural tolerance rather than sensitisation.

The nature of the deficit produced in the current study involved reference memory processes. However, it should be noted that both the binge MDMA treated and saline control groups produced more reference memory errors than working memory errors overall. Intuitively this makes sense as while subjects are learning the task they do not know which arms produce reinforcement and hence are likely to often enter arms that do not contain reinforcement. The subjects did not make many working memory errors at any stage during the initial and re-dosing phases of the study and hence appeared quickly able to learn not to re-enter the arms of the maze within a trial. However this may relate to the rats natural tendency to not repeat arms but instead alternate arm entries (Chrobak & Napier, 1992). Thus this finding replicates the results from first half of the current thesis that found MDMA administration affected reference memory processes more than working memory processes. In fact the finding that the reference memory deficit was not present until the rats had acquired the task is very similar to the results from the acute studies in the first half of the current thesis. Therefore it could be argued MDMA exposure did not disrupt learning processes but more likely memory processes as it disrupted information that had already been retained. As in the first half of the thesis this deficit could be due to a long-term memory impairment or a disruption in the rules of the task.

A limitation of the current study was the potential confound produced by the eight to ten week gap between administering the binge regime of MDMA and commencing maze training. Due to the evidence that an additional regime of MDMA was able to significantly disrupt performance in the current study the next experiment in the thesis aimed to examine what would happen to the ability to acquire the maze if the gap between receiving the same regime of MDMA and training was much smaller.

In conclusion this study found that an initial binge regime of MDMA failed to have any significant effect on acquiring the partially baited radial maze task. However, when an additional binge regime of MDMA was administered it produced a transient but significant decrease in accuracy and an increase in trial completion time compared to saline controls. During the re-dosing phase the number of working memory errors made did not significantly differ compared to the initial acquisition phase. During the re-dosing phase the MDMA treated group produced an increase in the number of reference memory errors made compared to the controls. Therefore when there was evidence of an impairment it appeared to be due to a deficit in reference memory performance. However, it is unclear as to whether the nature of this deficit involves learning processes or memory processes due to the deficit occurring after task acquisition has taken place.

During the acute phase of the task when rats received saline there appeared no difference in performance between the chronic MDMA and control group. However when rats were administered acute challenges of MDMA the control group showed greater impairment than the binge MDMA group. In addition during the acute challenge of MDMA, both groups produced more reference memory errors than working memory errors thus replicating the findings from the first half of this thesis. Therefore it would appear that binge MDMA exposure produces behavioural tolerance when later challenged with acute exposure to the drug.

Study 4: Binge Effects of MDMA on Acquisition and Reversal Learning in the Radial Arm Maze.

Initial acquisition

The rats in the first binge study of this thesis (Study 3) failed to show impairment in the acquisition of the partially baited radial arm maze after being exposed to an initial binge regime of MDMA. However these rats were not assessed in the radial arm maze until approximately eight to ten weeks after MDMA administration. There is physiological and behavioural evidence that the effects of binge MDMA treatment in rats are transient and recover over time (Scanzello et al., 1993; Sabol et al., 1996; Brennan & Schenk, 2006). In addition the findings of the previous study in this thesis found a significant impairment in the binge MDMA treated rats after they were exposed to an additional regime of MDMA. Thus the current study examined whether the reason there was no initial learning impairment shown by the MDMA-treated rats was due to the delay between drug exposure and training or whether the single binge MDMA regimen used in the previous study was not large enough to produce impairment.

The current study investigated what effect binge MDMA exposure would have on maze performance if a much shorter gap occurred between dosing and training. In the current experiment a new group of twenty rats was used to examine the effects of the previously used binge dose of MDMA on acquisition of the partially baited radial arm maze task. Again in this study ten rats were given a binge regime of MDMA while ten controls received saline injections. However, in the current study the rats were already at 85% of their free feeding body weight and had received the habituation

phase of training before the drugs were injected. Maze training then began two days after MDMA and saline were administered.

Drug Tolerance versus Sensitisation

In Study 4 preliminary evidence of drug tolerance was found as rats that had received two binge regimes of MDMA were less affected by a subsequent acute dose of MDMA compared to saline controls that had not previously been exposed to MDMA. Therefore the current study also attempted to replicate and extend this finding by administering acute doses of MDMA and saline to the rats after they had acquired the radial arm maze task after only being exposed to a single binge regime of MDMA.

Reversal Training

Research examining the effects of binge MDMA exposure on cognition has found evidence of long-term learning impairments (Broening et al., 2001; Williams et al., 2003; Sprague et al., 2003; Vorhees et al., 2004; Skelton et al., 2006; Able et al., 2006; Skelton et al., 2008; Skelton et al., 2009). In addition to learning impairments there is evidence that MDMA exposure may impede the ability of subjects to adapt their behaviour to changing consequences. For example the Wisconsin Card Sorting Task (WCST) utilises a constant changing of task rules that is used to accesses cognitive flexibility and it has been found that Ecstasy users are impaired on this task (von Geusau et al, 2004; Smith et al., 2006). Similarly tasks that assess associative learning have been found to be impaired due to perseverative responding in Ecstasy users (Montgomery et al., 2005).

More recently Dafters (2008) used a modified Stroop task to examine task switching in Ecstasy users. In a standard Stroop task participants are presented with word stimuli that are the names of colours displayed in different coloured ink and participants have to report the colour that the word is presented in (Dafters, 2008). This involves having to resist reading the word and subjects tend to be faster when the name of the word and the ink match and take longer when they do not (Dafters, 2008). In the modified task on random trials the presented word was underlined indicating that in this trial participants were required to name the word rather than the ink colour, hence requiring a switch in task. Ecstasy users were significantly slower than controls during the task switching trials and impairment was correlated with amount of previous Ecstasy use. The finding that participants with a history of Ecstasy use are impaired on this Stroop switching task has also been found by Lamers, Bechara, Rizzo and Ramaekers (2006).

Within the animal literature there is also evidence that reversal learning is impaired after MDMA exposure. For example Verrico, Lynch, Fahey, Fryer, Miller and Madras (2008) found MDMA administered both orally and intramuscularly to cynomolgus monkeys significantly disrupted performance on a well learnt reversal learning task. This was evidence that acute MDMA exposure produced perseverative errors indicating MDMA administration can impair the ability to suppress irrelevant information and adapt behaviour in the face of changing consequences (Verrico et al., 2008).

Possibly more relevant to the current study is that MDMA treated animals have trouble adapting their behaviour to changing consequences in studies utilising reversal phases in the Morris water maze. In this paradigm once rats have acquired the task and learnt the position of the platform it is shifted and the ability of the rats to learn its new location is assessed (Morris, 1984). There have been several studies indicating chronic

or binge MDMA exposure produces impairments in these tasks. In Williams et al.'s (2003) study once initial Morris water maze acquisition occurred the platform was shifted to the opposite quadrant of the pool. Once performance had stabilised during this reversal phase the platform was reduced in size and moved again. Rats that received a chronic regime of MDMA produced significantly longer latencies, greater path lengths and cumulative distance from the platform during both of these reversal phases suggesting that MDMA significantly impaired their ability to alter their behaviour to changing task demands. More recently Skelton et al. (2006) and Skelton et al. (2009) also found that administering a chronic MDMA regime to rats significantly impaired their performance during a reversal phase and a reduced platform reversal phase.

However not all studies using Morris water maze reversal phases have found such clear MDMA induced impairments. For example Skelton et al. (2008) found MDMA treatment did not impair performance during an initial reversal phase but did impair performance on an additional reduced platform reversal phase suggesting a task switching deficit. Able et al. (2005) found no difference in performance between rats exposed to a binge regime of MDMA and those given saline during acquisition of a reduced platform reversal phase. However, during probe trials that were conducted afterwards where the platform was removed, the MDMA treated rats showed a significantly greater average distance from where the platform had been indicating some degree of impairment in reversal learning.

In conclusion there is converging evidence from human Ecstasy studies and experimental animal research suggesting MDMA exposure produces deficits in changing behaviour when tasks are altered. Therefore in the final phase of the current study the ability of the binge MDMA treated and saline control rats to adapt their performance to changing consequences was investigated. This was assessed by

reversing the rules of the maze task whereby the arms that had initially contained reinforcement were changed. Therefore, the arms of the maze that previously had not contained reinforcers would now have reinforcers while the arms that previously contained reinforcement would now not have reinforcers.

The Current Investigation

The present study examined the effect of a binge regime of MDMA on acquisition in the partially baited radial arm when training commenced two days after drug administration. It was hypothesised that the MDMA treated rats would show evidence of learning impairments compared to saline controls and that this impairment would be predominantly evident by MDMA treated rats producing more reference memory errors than controls. The current study also utilised a reversal phase where the reinforced arms of the maze were swapped to assess the effects of a binge MDMA regime on a task requiring subjects to alter their behaviour. It was hypothesised that MDMA treated rats would be impaired in their ability to adapt their behaviour compared to saline controls which would be evident by MDMA treated rats acquiring the new task more slowly than controls.

In between the acquisition and the reversal phases, acute doses of MDMA and saline were administered to examine whether behavioural tolerance would be evident in rats that were previously exposed to MDMA. Based on the findings from the previous study of this thesis it was hypothesised that the previously treated binge MDMA rats would be less impaired when administered acute doses of MDMA compared to saline controls.

Method

Subjects

The subjects were twenty white male Sprague-Dawley rats that were approximately three to four months old at the beginning of the study. The rats were housed individually and were experimentally naïve at the beginning of the study. They were kept at 85-90% (between 218 and 324 grams) of their free feeding body weight and began habituation training around two weeks after reaching this weight. They had continuous access to water and were kept on a 12:12-hour dark:light cycle and were run during the light phase of this cycle.

Apparatus/Materials

The maze and reinforcers used were the same as those stated in the General Acute MDMA Method section. Drugs used were saline 0.9 % and MDMA 10 mg/kg, which were prepared on the day of use by dissolving to the required dose in 0.9 % saline solution. During the tolerance phase of the experiment MDMA 4.0 mg/kg was used and it was also dissolved to the required dose in 0.9 % saline solution.

Procedure

The current study used a between-subjects experimental design where one group of rats received binge doses of MDMA and the other received saline. Rats were assigned into the experimental and control group (10 rats in each group). Unfortunately one of the binge rats died after receiving MDMA and therefore, there were only nine rats in the binge group, compared to ten in the saline control group. Again each rat was assigned a set of four reinforced arms and four non-reinforced arms within the maze. The binge and control rats had the same sets of reinforced and non-reinforced arms, so

that the first rat in both groups had the same reinforced arms and so that there were matched pairs of rats. This was done to control for difficulty of the task in case some sets of maze arms were easier to learn that others.

Training was conducted in the same way as outlined in the General Binge MDMA Method section. However, in this study the rats received the habituation pre-training before they were given the injections. The day after completing the habituation phase, rats were given four injections of either 10 mg/kg of MDMA or 0.9 % saline. The following day rats were given a rest day. Training involving assigned reinforcer arms began after the rest day (two days after the injections). Unlike the previous study rats were not run within their experimental groups but in their matched pairs. This was done as it was more convenient in terms of not having to change the arms that needed to be baited so often. However, to control for order or odour scenting in the maze the running was counterbalanced so that which rat ran first was changed on alternate days so one day the binge rat in the pair would run first and the next day the control rat was run first.

Each group of rats was given five sessions of training per week. In the first phase of the experiment both groups of rats had to reach a criterion of a group average of at least eight five percent accuracy for six consecutive days before they had been considered to achieve acquisition. This took twenty four training sessions and occurred 30 days after drug treatment.

Two days (32 days post drug treatment) after the first phase was completed the second phase began. This second phase examined the effects of acute challenges of MDMA after the rats had acquired the radial maze task. During this phase each rat from the previous experimental and control groups received all drugs and doses. As this study was conducted in between the acquisition and reversal phases of the

previous experiment each rat used its original set of four reinforced arms. Maze running was identical to that explained in the general method section. Each rat was injected i.p. twenty minutes before running. Rats were run in batches as described previously. Each drug dose was repeated; therefore two doses of 4 mg/kg MDMA and 0.9% saline were administered to each rat. Several days were left in between each drug treatment to control for carry on effects of the drugs. On these days the rats were trained in the maze without being administered drugs.

To ensure that no residual acute drug effects were present the final phase of the experiment began three days after completion of the second phase and hence began 46 days post drug treatment. During the final phase of the experiment the effect of changing the rules of the task was assessed. During this third phase each rat's previously reinforced maze arms now did not contain obtainable reinforcers and the previously non-reinforced arms now contained obtainable reinforcers. Rats continued to run as in the first phase of the experiment where they were allowed to enter four arms of the maze per trial and received three trials of training per day. As in the first phase rats were run in their matched pairs and the order in which the rats within the pairing ran was counterbalanced as before. Training continued until a 90% level of accuracy for both groups was achieved. During this reversal phase this took eighteen training sessions which concluded 70 days post binge drug treatment.

Results

In all figures error bars show standard error of the mean. Daily percent correct figures were calculated by averaging across the three daily trials to obtain an average level of performance for the session for each individual rat. Group averages were then

calculated to obtain a group mean for each group and acquisition session. These data are presented below in Figure 18 and show during initial acquisition both the MDMA group and the saline control group began the task with an accuracy level of approximately fifty percent (which is above chance in this task) and this gradually increased across the sessions until rats were performing consistently above ninety percent.

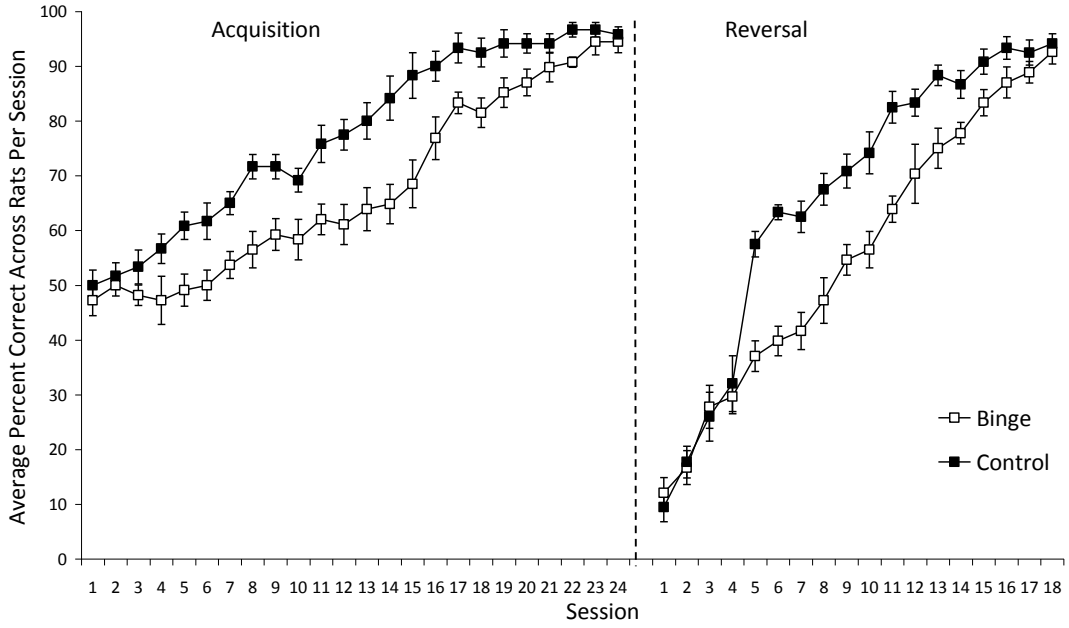

Figure 18: Average percent correct per session across all rats in both the binge MDMA group and the saline control group for acquisition and reversal training.

Although performance of the two groups starts at a similar level for the first three sessions, the saline group's performance seems to improve at a faster rate than the MDMA group. However, by the last two sessions of training the performance of the two groups is similar. This suggests the group that received the binge regime of MDMA acquired the task slower than the saline control group, but did manage to eventually perform at similar levels to the control group. These effects were confirmed

by a two-way mixed ANOVA. Main effects for session, $F(23, 391) = 104.89$, $p < 0.05$ ($p = 0.00$) and group, $F(1, 17) = 16.23$, $p < 0.05$ ($p = 0.00$) were found but were moderated by a significant interaction between session and group, $F(23, 391) = 2.90$, $p < 0.05$ ($p = 0.00$).

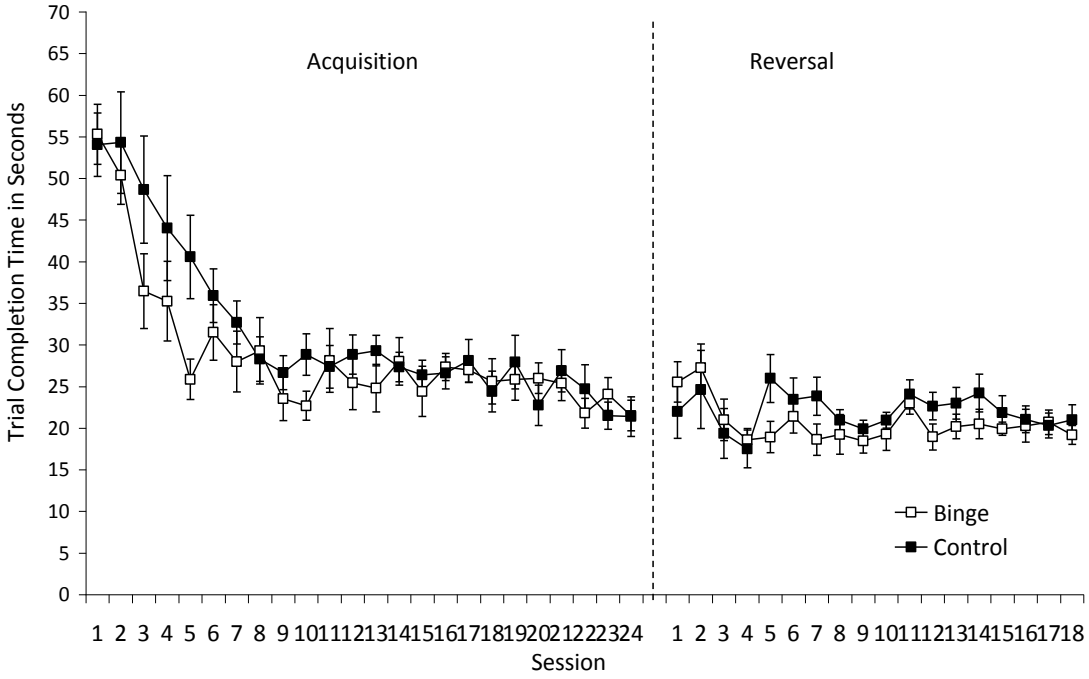

Figure 19: Average trial completion time in seconds per session across all rats in the binge MDMA and the saline control group for acquisition and reversal training.

During the reversal phase of the experiment Figure 18 shows initially both groups were very inaccurate producing an average of around 10% correct. Again, this gradually increased for both groups over the training sessions with performance on the last session reaching around 90% correct. As in the acquisition phase both groups produced similar levels of accuracy during the initial training sessions. However, on the fifth session the control group's performance dramatically increased and continued progressing at a faster rate than that of the MDMA treated group. Finally on the last

two sessions the two groups performances were similar. This trend suggests the group that were administered MDMA acquired the reversal task at a slower rate than that of the saline controls. However once again they were eventually able to perform at a similar level to the controls by the end of training. Again these effects were corroborated with a significant interaction between session and group $F(17, 289) = 4.52$, $p < 0.05$ ($p = 0.00$). There was also a main effect for session, $F(17, 289) = 184.859$, $p < 0.05$ ($p = 0.00$), and group, $F(1, 17) = 31.81$, $p < 0.05$ ($p = 0.00$).

Mean trial completion times in seconds for each daily session, were also calculated for each rat. Group means were then calculated for the MDMA treated group and the control group. These data are depicted in Figure 19 and show that trial completion times for both groups during the initial acquisition phase of the experiment decreased as training continued which was confirmed by a main effect for session, $F(23, 391) = 26.02$, $p < 0.05$ ($p = 0.00$). It also depicts that trial completion times between the two groups were similar. A two-way mixed ANOVA revealed a significant interaction between group and session, $F(23, 391) = 1.69$, $p < 0.05$ ($p = 0.03$) and no main effect for group, $F(1, 17) = 0.76$, $p > 0.05$ ($p = 0.40$).

Despite showing decreases in accuracy during initial learning as seen in Figure 18 there does not seem to be any obvious effect of changing the task on trial completion time as shown in Figure 19. There also does not seem to be any difference in trial completion times between the two groups during the reversal phase. Therefore although rats become much less accurate during the reversal phase the average time it took them to complete a trial did not seem altered apart from maybe a slight increase in trial completion time during the first couple of trials. These effects was supported as no significant interaction between group and session was found, $F(17, 289) = 0.64$, $p > 0.05$ ($p = 0.86$). There was also no main effect for group, $F(1, 17) = 1.24$, $p > 0.05$ (p

= 0.28). The ANOVA revealed a main effect for session, $F(17, 289) = 3.78$, $p < 0.05$ ($p = 0.00$) but there was no consistent trend in the data over sessions.

Working Vs. Reference Memory Data

To examine the number and patterns of errors produced, the number of working memory and reference memory errors made per session for each rat was recorded. These figures were then converted into percentage error values. Average error percentage values were calculated for both the binge MDMA group and the saline control group by averaging across the rats in these groups. This was done for each daily session for both the acquisition and the reversal phases of the study.

To analyse the error data a 2-way repeated measures ANOVA was conducted on the acquisition data comparing error type and training. During the acquisition phase a significant interaction was found between error type and session, $F(23, 414) = 35.09$, $p < 0.05$ ($p = 0.00$). Therefore there was a significant difference in the type of errors made across the training sessions during task acquisition. During the reversal phase a significant interaction between error type and session was also found, $F(17, 306) = 95.30$, $p < 0.05$ ($p = 0.00$). When comparing the working memory data on Figure 20 and the reference memory data on Figure 21 it is obvious that more reference memory errors were made than working memory errors during both acquisition and reversal phases of the study. These effects were examined further by individually analysing the data for working and reference memory errors across training sessions and between groups.

The data for average working memory errors is presented in Figure 20. This figure shows that there were very few working memory errors made during

acquisition. The saline control group made more working memory errors than the MDMA binge group during the first three training sessions but on subsequent training sessions there appears no obviously trend in the data. A 2-way mixed ANOVA revealed a significant interaction between session and group, $F(23, 391) = 2.46$, $p < 0.05$ ($p = 0.00$). This indicated that there was a significant difference between two groups in the number of working memory errors made and this was dependent on training session. During acquisition there was no main effect for session, $F(23, 391) = 0.92$, $p > 0.05$ ($p = 0.58$) and no main effect for group, $F(1, 17) = 0.01$, $p > 0.05$ ($p = 0.93$).

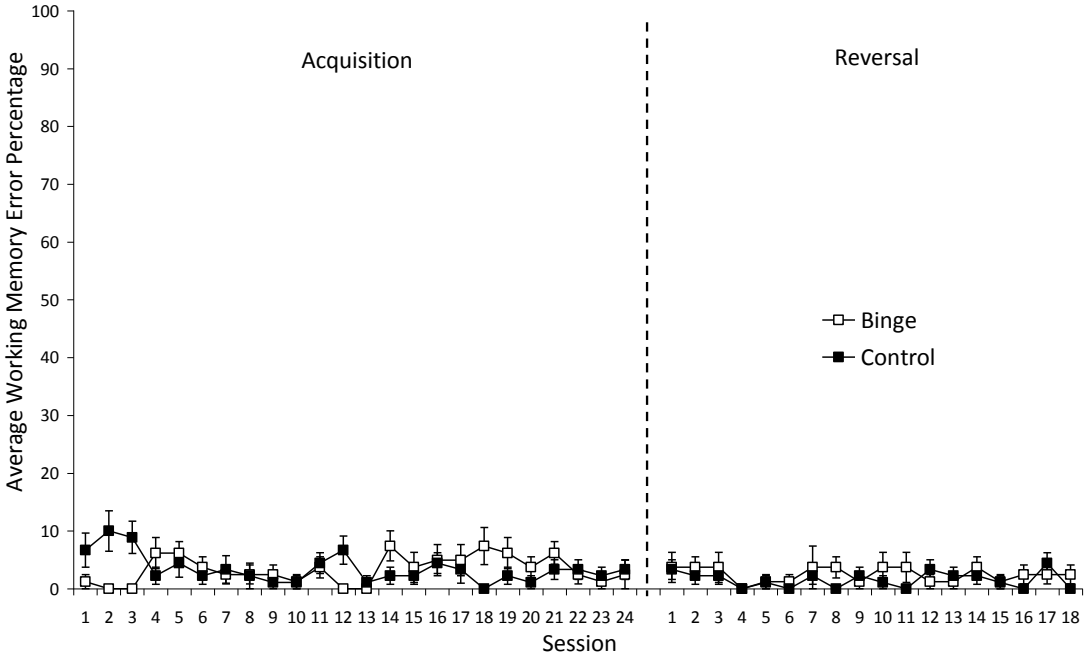

Figure 20: Average working memory error percentages across all rats in both the binge MDMA and saline control groups during acquisition and reversal training.

During the reversal phase of the study both groups made very few working memory errors with no clear differences between the two groups. Also the number of

working memory errors remained fairly stable across the entire reversal phase. This was confirmed by a 2-way mixed ANOVA that found no significant interaction between session and group, $F(17, 289) = 0.63$, $p > 0.05$ ($p = 0.87$). There were also no main effects for session, $F(17, 289) = 0.83$, $p > 0.05$ ($p = 0.65$) and group, $F(1, 17) = 1.80$, $p > 0.05$ ($p = 0.20$).

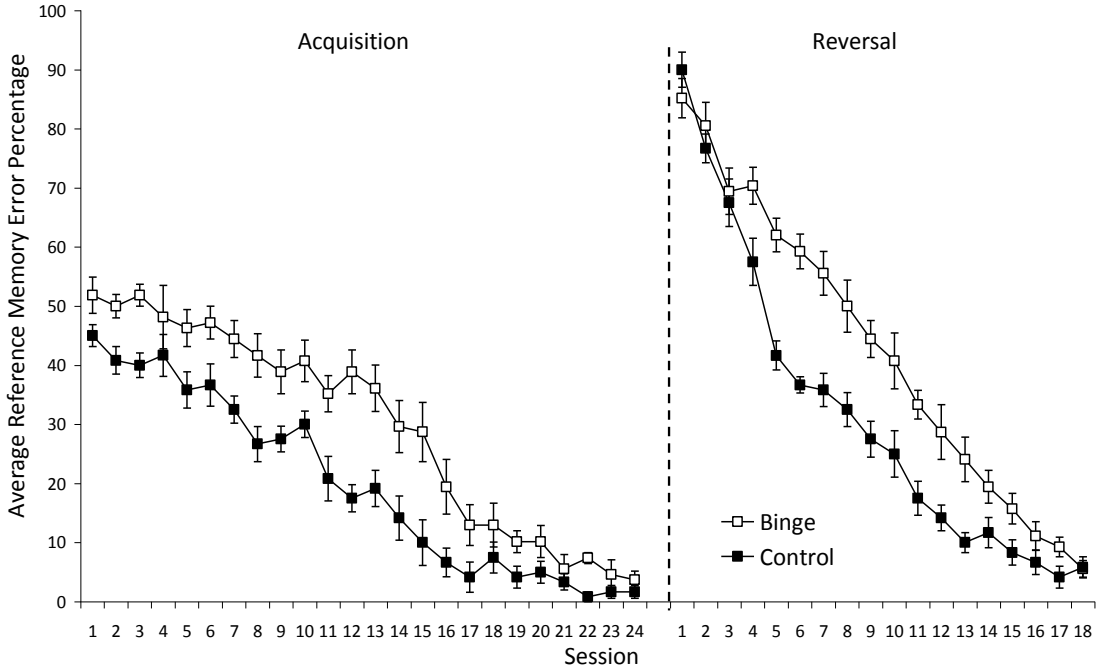

Figure 21: Average reference memory error percentages across all rats in both the binge MDMA and saline control groups during acquisition and reversal training.

Data depicting reference memory performance are shown in Figure 21 and show that both groups made a lot of reference memory errors. During the acquisition phase both groups made a large number of reference memory errors at the beginning of training and this steadily decreased over the training sessions as the task was acquired. By the end of the training session both groups were making similar numbers of reference memory errors. During the acquisition phase the saline control rats appeared

to reduce the number of reference memory errors made across sessions at a faster rate than the binge MDMA treated rats. These effects were corroborated by a 2-way mixed ANOVA that found main effects for session, $F(23, 391) = 84.91$, $p < 0.05$ ($p = 0.00$) and group, $F(1, 17) = 17.84$, $p < 0.05$ ($p = 0.00$) which were moderated by a significant interaction between group and training session, $F(23, 391) = 2.20$, $p < 0.05$ ($p = 0.00$).

During the reversal phase both groups made a very high number of reference memory errors during the first couple of sessions. The number of reference memory errors than steadily decreased as the subjects began to adapt their behaviour to the change in the task. However, the control rats were quicker to do this than the MDMA treated group as shown by the faster decrease in the number of reference memory made by the saline controls. By the end of the reversal phase the MDMA group and the saline controls were producing a similar number of reference memory errors where both groups were making very few reference memory errors. These effects were confirmed by a significant interaction between group and training, $F(17, 289) = 4.13$, $p < 0.05$ ($p = 0.00$). During the reversal phase there was also a main effect for session, $F(17, 289) = 171.76$, $p < 0.05$ ($p = 0.00$) and a main effect for group, $F(1, 17) = 29.64$, $p < 0.05$ ($p = 0.00$).

Acute Data:

Percent correct figures were calculated in the same way as in the previous acute drug studies. Group values for the binge MDMA treated rats and saline controls were then calculated for each drug session and these values were averaged across the two sessions of each acute drug treatment (MDMA and saline). This data is shown in

Figure 22 and shows both the binge MDMA group and the saline controls produced a very high level of accuracy during acute saline administration. There also appears to be no difference in accuracy between the two groups during saline exposure.

With the acute administration of MDMA both groups showed a marked impairment with a large drop in accuracy where the saline control group appears to be more affected by the acute administration of MDMA than the binge MDMA group. This effect was supported by a 2-way mixed ANOVA with a significant interaction between group and drug treatment, $F(1, 17) = 9.72$, $p < 0.05$ ($p = 0.01$). There was also a main effect for acute drug treatment, $F(1, 17) = 213.33$, $p < 0.05$ ($p = 0.00$) but no main effect for group, $F(1, 17) = 3.62$, $p > 0.05$ ($p = 0.07$).

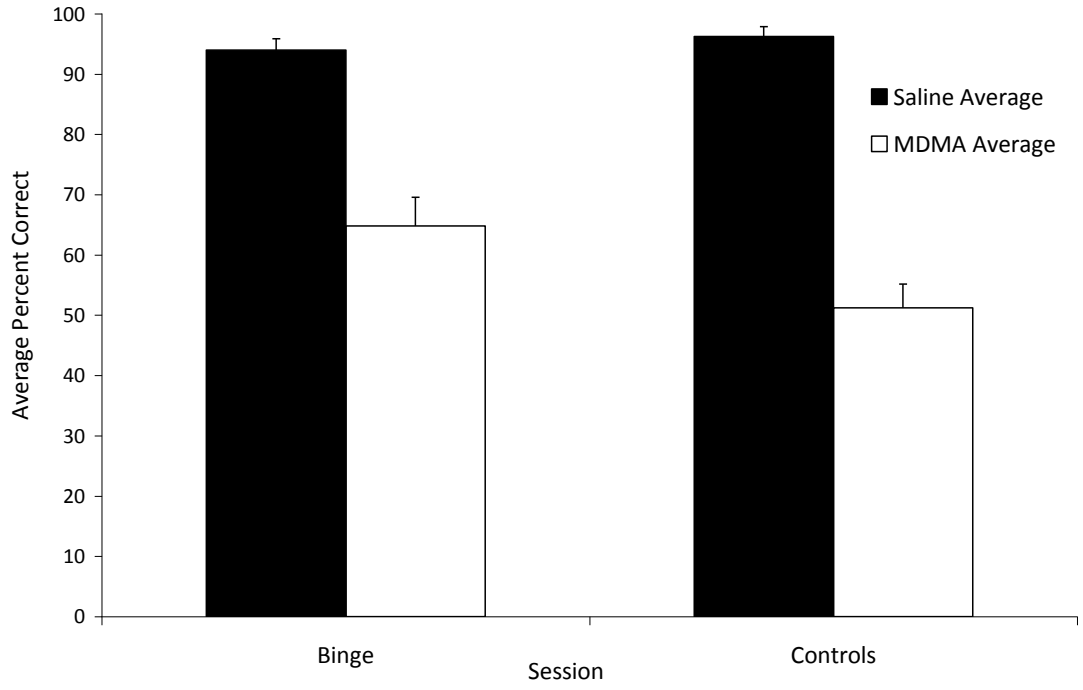

Figure 22: Average percent correct across all rats in both the binge MDMA group and the saline control group for the acute doses of saline and MDMA.

Average session trial completion times (in seconds) were calculated for each individual rat and were used to obtain group averages. These data are depicted in Figure 23 and show that both the binge MDMA and saline control groups produced similar low trial completion times during the saline sessions. However, during the acute MDMA sessions both groups produced much longer trial completion times compared to the saline sessions. The saline control group produced only a slightly longer average trial completion time than that of the binge MDMA group suggesting they were not obviously more affected than the MDMA binge group when administered with acute doses of MDMA.

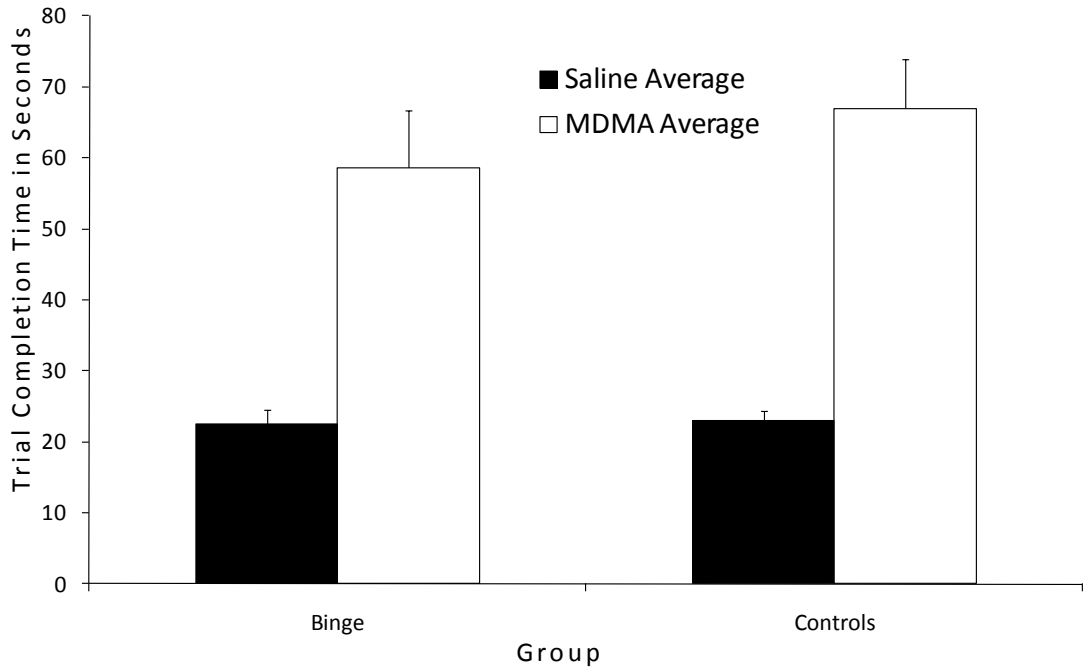

Figure 23: Average trial completion time in seconds across all rats in both the binge MDMA group and the saline control group for the acute doses of saline and MDMA.

This lack of an effect was confirmed by a 2-way mixed ANOVA where no significant interaction between acute drug treatment and group was found, $F(1, 17) =$

1.26, $p > 0.05$ ($p = 0.28$). There was also no main effect for group, $F(1, 17) = 1.38$, $p > 0.05$ ($p = 0.26$). There was a main effect for drug treatment, $F(1, 17) = 108.65$, $p < 0.05$ ($p = 0.00$) and from Figure 23 it is obvious that acute MDMA treatment impaired performance more than acute saline treatment.

Differences between error types were examined by calculating the number of working and reference memory errors made per session for each rat. These figures were then converted into percentage error values. Average error percentage values were calculated for both the binge MDMA group and the saline control group by averaging across the rats in these groups and are depicted in Figure 24.

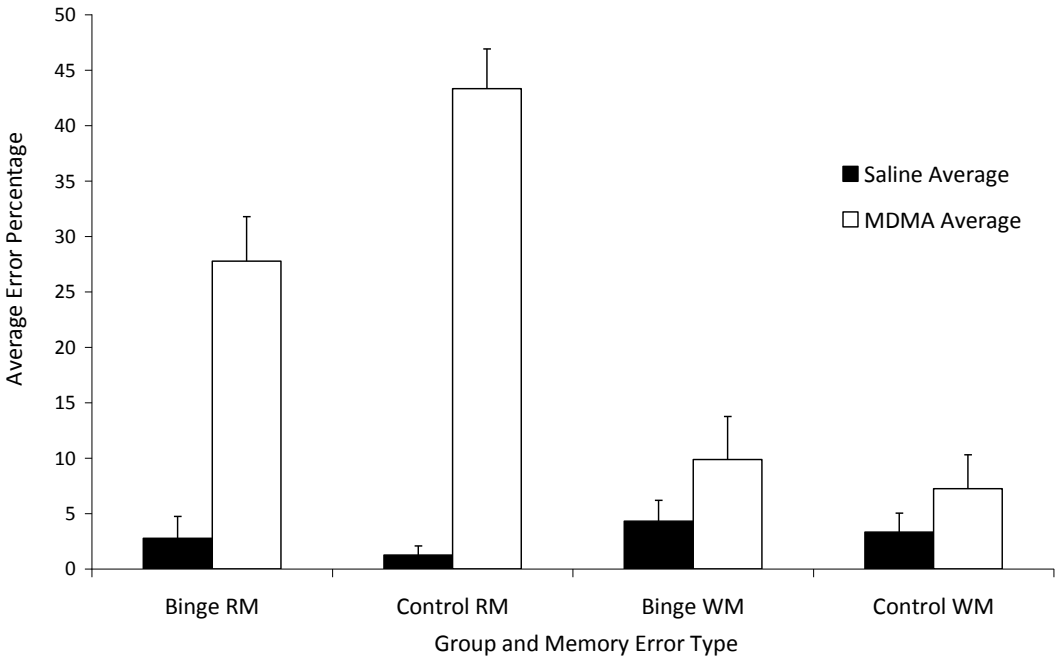

Figure 24: Average error percentage of working memory (WM) and reference memory (RM) errors across all rats for both the MDMA binge group and the saline control group.

When examining the working memory error data Figure 24 shows that during the saline session very few working memory errors were made by either the binge

MDMA group or the saline control group. Also the number of working memory errors made during saline treatment did not seem to obviously differ between the two groups. With acute exposure to MDMA the number of working memory errors increased slightly for both groups and the binge MDMA group produced marginally more working memory errors than the control group. These small effects were shown to be non significant using a 2-way mixed ANOVA that revealed no interaction between acute drug treatment and group, $F(1, 17) = 0.13$, $p > 0.05$ ($p = 0.72$) and no main effects for acute drug treatment, $F(1, 17) = 4.30$, $p > 0.05$ ($p = 0.05$) or group, $F(1, 17) = 0.61$, $p > 0.05$ ($p = 0.45$).

Figure 24 also shows that very few reference memory errors were made by either group during the saline session. During the acute MDMA session there were a large number of reference memory errors made by both groups. In addition the saline control group appeared to make more reference memory errors than the binge MDMA group when administered acute doses of MDMA. These effects were confirmed by a 2-way mixed ANOVA that produced a significant interaction between acute drug treatment and group, $F(1, 17) = 19.11$, $p < 0.05$ ($p = 0.00$). There were also main effects for acute drug treatment, $F(1, 17) = 294.46$, $p < 0.05$ ($p = 0.00$) and group, $F(1, 17) = 6.25$, $p < 0.05$ ($p = 0.02$).

To compare error types a 3-way mixed ANOVA was conducted examining memory type (working and reference memory errors), acute drug treatment (MDMA or saline) and group (binge and control). There was a 3-way interaction between memory type, acute drug treatment and group, $F(1, 17) = 9.36$, $p < 0.05$ ($p = 0.01$). Thus the type of error made differed significantly depending on the type of acute drug administered and whether the subjects had been pre-treated with binge MDMA or saline. Referring to Figure 24 this result suggests there were more reference memory

errors than working memory errors during acute MDMA administration and this was more pronounced in the saline control group.

There was also an interaction between memory type and group, $F(1, 17) = 4.81$, $p < 0.05$ ($p = 0.04$). Thus there was a significant difference in the type of errors made between the two groups. Examining Figure 24 shows the control group made more reference memory errors than the binge group. In addition there was a significant interaction between memory type and acute drug treatment, $F(1, 17) = 88.42$, $p < 0.05$ ($p = 0.00$). Hence there was a significant difference in the type of memory errors made during acute MDMA and saline treatments where MDMA treatment produced more reference memory than working memory errors. Finally there was a significant interaction between acute drug treatment and group, $F(1, 17) = 6.90$, $p < 0.05$ ($p = 0.02$), indicating a significant difference on the effects of administering acute doses of MDMA and saline on group performance. Main effects for memory type, $F(1, 17) = 39.13$, $p < 0.05$ ($p = 0.00$) and acute drug treatment, $F(1, 17) = 169.96$, $p < 0.05$ ($p = 0.00$). There was no main effect for group, $F(1, 17) = 2.58$, $p > 0.05$ ($p = 0.13$).

Discussion

Acquisition and Reversal

To recap, the aim of this study was to examine if a binge dose of MDMA would impair acquisition of the partially baited radial arm maze task if training commenced two days after drug administration. During the initial acquisition phase both groups of rats learnt the task as accuracy (percent correct) significantly increased during training. During this phase the binge MDMA group showed evidence of learning impairment compared to controls as they acquired the task at a significantly

slower rate. However, they were able to eventually acquire the task and perform at a similar level to the saline controls. Throughout the acquisition phase trial completion time significantly decreased for both groups of rats as they learnt the task. Of note there was no significant difference between the two groups on this variable suggesting that the learning impairment produced by MDMA exposure was not the result of a motor impairment.

In terms of the types of error made, during acquisition both groups produced a similar low number of working memory errors. During training both groups initially produced a high number of reference memory errors and as this phase continued the MDMA treated group made significantly more reference memory errors than the controls. Again by the end of training performance was similar between the two groups with both of them producing a low number of reference memory errors. Therefore MDMA exposure produced evidence of a learning impairment and this cognitive deficit appeared to involve reference memory processes.

This finding is consistent with previous literature that has utilised Morris and Cincinnati water maze tasks that have found that chronic or binge MDMA exposure impairs the acquisition of tasks requiring reference memory processes (Robinson et al., 1993; Broening et al., 2001; Williams et al., 2003; Sprague et al., 2003; Vorhees et al., 2004; Skelton et al., 2006; Able et al., 2006; Skelton et al., 2008; Skelton et al., 2009). However it does conflict with findings from more operant based tasks that have failed to find evidence of learning impairments (Li et al., 1988; Byrne et al., 2000; Winsauer et al., 2002; Moyano et al., 2005). Again a possible reason why these chamber-based operant tasks failed to show evidence of impairment following MDMA administration could be due to the tasks not having a large reference memory component compared to the maze tasks that have found evidence of MDMA induced cognitive impairments. Another possible explanation for the conflicting findings between operant chamber

tasks and maze paradigms is that the latter involves greater spatial memory processes which may be more susceptible to the effects of MDMA exposure.

The findings from the previous study of this thesis that failed to find evidence of binge MDMA exposure impairing task acquisition can be addressed using the results from the current study. Of note, the finding that the same dose as that used in the previous study was able to impair task acquisition suggests the lack of deficit found in the previous study was not due to the size of the drug regimen used. An alternative explanation for the conflicting findings is due to the delay between drug exposure and training. The current study found that a binge regime of MDMA was able to produce a learning deficit in the partially baited radial arm maze when only two days had elapsed between drug exposure and training. This finding suggests that the failure of the previous study to find a learning deficit could be due the long period of time (approximately eight to ten weeks) between drug administration and commencement of training. In addition the fact that for the first couple of trials the two groups of rat's performance did not differ can rule out the explanation that any difference seen were due to residual acute effects of the drug treatment. Therefore the seemingly most plausible explanation for the failure to find evidence of an acquisition impairment following MDMA exposure in the previous study is because the large time gap between drug exposure and training allowed a degree of recovery of function to occur. There is some evidence within the neurochemical literature to support this hypothesis as the damage to 5-HT levels produced by the same regime of MDMA as that used in the current study has been shown to recover over time (Scanzello et al., 1993). However as our study did not assess 5-HT levels during the study we can directly provide evidence of physiological recovery from the effects of MDMA exposure.

The current experiment also examined if a binge dose of MDMA would affect the ability of rats to adapt their performance to changing consequences when the rules

of the radial arm maze paradigm were reversed. During the reversal phase the saline controls learnt the task significantly faster than the MDMA treated group. Thus MDMA exposure significantly impaired the ability of the rats to adapt their behaviour to a change in task demands. However by the end of training both groups produced a similar level of performance indicating that eventually the MDMA group were able to acquire the new task. Also in this phase there was no real increase in trial completion time for both groups and this did not differ between the two groups. Therefore although both groups of rats were initially less accurate during the task change they did not take longer to complete trials during this phase of the task. While MDMA exposure impaired accuracy it did not significantly affect the amount of time it took to complete trials and hence the deficit seen in this phase cannot be explained in terms of motor impairments.

When examining the kind of errors made both groups during the reversal phase they produced very few working memory errors and this did not differ significantly between the two groups. In contrast the reversal phase produced a large increase in reference memory errors for both groups. During this phase the MDMA treated group produced significantly more reference memory errors than the saline controls. Hence once again the impairment produced by MDMA exposure involved reference memory processes. However, it should be noted that while entering non-reinforced arms in this phase was counted as a reference memory error the same way as in the previous phase the cognitive process they were assessing may be different. This is because reference memory errors in the reversal phase meant subjects were failing to alter their behaviour rather than remembering previously learnt information about which arms contained reinforcement.

The finding that binge MDMA exposure produced significant impairments in cognitive flexibility is consistent with previous literature (von Geusau et al., 2004;

Smith et al., 2006; Montgomery et al., 2005; Dafters, 2008; Lamers et al., 2006) that has found that Ecstasy users are impaired at tasks that involve altering their behaviour in response to changing task demands. In addition it is consistent with research (Williams et al., 2003; Able et al., 2005; Skelton et al., 2006; Skelton et al., 2008; Skelton et al., 2009) that has utilised reversal phases in the Morris water maze which require subjects to alter their behaviour once the initial task had been acquired. Again there is some neurochemical evidence within the literature to further support these findings. For example there is evidence that monkeys produce significant perseverative impairments on reversal discrimination tasks following experimental 5-HT depletion in the prefrontal cortex (Clarke, Dalley, Crofts, Robbins & Roberts, 2004; Clarke, Walker, Dalley, Robbins & Roberts, 2007). As the binge regimen of MDMA utilised within the current study has been shown to significantly reduce 5-HT levels in multiple brain regions including the prefrontal cortex (Scanzello et al., 1993) this may account for the finding that MDMA exposure impairs cognitive flexibility. Again as this study did not conduct any physiological measures of 5-HT activity it is unknown as to the degree of 5-HT depletion subjects in the current study experienced.

In conclusion rats administered a large binge dose of MDMA took significantly longer to learn both the acquisition and reversal task in the partially baited radial arm maze compared to saline controls. However, they were able to eventually acquire both tasks performing at a similar level to saline controls. In addition when there was evidence of impairment it appeared to primarily involve reference memory processes. The deficits seen appeared to be relatively long-term as the subjects continued to show impairment during the reversal phase of the task which began 46 days after drug treatment and did not reach a similar level of performance to controls till around 68 days post drug treatment. Therefore while performance of the MDMA treated animals appeared to be similar to the saline controls by the time acquisition training was

finished, the fact they then showed impairment when they were required to alter their behaviour suggests they still were suffering from an underlying cognitive impairment. Hence one possible reason why some research has failed to show evidence of cognitive impairments produced by MDMA exposure is that they have involved tasks where the subjects have already received a lot of training on the task. The current study would suggest that an impairment would be more visible on tasks that were still being acquired and those that required subjects to alter their behaviour.

There are several potential explanations for the underlying cause of these cognitive impairments. The impairments produced by binge MDMA exposure in the current study are characterised as learning impairments, hence subjects that received MDMA had trouble acquiring the task compared to controls. One possibility for this is that MDMA treatment may produce problems in consolidating the information about which arms contain reinforcement into long-term memory. Hence they have trouble learning the arms of the maze that contain reinforcers as this information takes longer to enter and become available in long-term memory. When the task changes and the reinforced arms of the maze are reversed, once again the rats have trouble encoding the new information about the arms into long-term memory.

Another possible explanation is that MDMA produces impairments in acquiring task rules. Therefore the reason why the subjects that received MDMA were slower to acquire the task is that it took them longer to ascertain that there were fixed arms that contained reinforcement and those that did not. This explanation would also explain the findings from the reversal phase as MDMA administration could have then impaired the subjects ability to ascertain that the rules of the task had changed and then impaired their ability to acquire the new task rule. Unfortunately the current study does not allow us to differentiate between these two possible explanations as to the underlying cognitive impairment seen with MDMA exposure.

A possible confound in the current study was that the acute drug phase of the experiment was run in between the acquisition and reversal phases. There should be no residual acute effects of the drug during the reversal phase because of the three day gap between the last acute MDMA drug session and the beginning of the reversal phase. However, there is the possibility that the acute dose of MDMA may have in some way affected performance during the reversal phase of the task. For example, it is unknown what effect two additional acute doses of MDMA would have on the rats that had already received a binge regime of MDMA. While there is evidence that there is both behavioural (Brennan & Schenk, 2006) and neurochemical (Scanzell et al., 1993) recovery of function after the binge regime of MDMA used in the current study, it is unknown what effect additional low doses of acute MDMA would have on this process. Therefore, there is the possibility that these additional acute doses of MDMA may have affected performance in the reversal phase. Future studies could replicate this experiment by excluding the acute drug treatments between the acquisition and reversal phases of the study to examine whether this factor would influence performance.

Acute MDMA Effects

To reiterate, the aim of this phase of the study was to examine whether rats that had already acquired the radial arm maze task and had been exposed to a binge dose of MDMA would show evidence of behavioural sensitisation or tolerance when exposed to acute administration of MDMA compared to saline controls. During acute saline administration both the binge MDMA and control groups produced a high level of accuracy and there was no real difference between the groups. However, during acute MDMA administration both the binge MDMA and control groups produced a decrease

in accuracy. Also, the control group was significantly more impaired than the binge MDMA treated group. There were no real differences between the groups in terms of trial completion time when saline was administered, but during the acute MDMA phase both groups trial completion times increased and there was no significant difference between the two groups.

The control group produced more reference memory errors than the binge MDMA group, but the groups produced similar amounts of working memory errors. Therefore, we found evidence of behavioural tolerance as the saline controls who had not received the binge MDMA regime performed worse when administered acute MDMA. The impairment produced by acute MDMA exposure involved reference memory processes replicating the acute effects from the first half of the thesis. The finding that behavioural tolerance occurred is consistent with previous research (Shankaran & Gudelsky, 1999; Piper et al., 2006; Brennan & Schenk, 2006; LeSage et al., 1993; Marston et al., 1999; Frederick et al., 1995; Frederick & Paule, 1997; Frederick et al., 1998) and the findings from the previous study of this thesis.

Conclusion

To summarise, the current study found when rats were treated with a binge regime of MDMA and began training shortly after drug exposure they took significantly longer and had a slower rate of learning compared to saline controls when acquiring the partially baited radial arm maze. As subjects were able to eventually acquire the task and perform at a similar level to that of the controls it appeared this impairment was transient. However, when the rules of the task were changed the MDMA treated rats were again significantly slower to adjust their behaviour and learn

to perform the new task. Therefore, this learning impairment appeared to be long-term in nature as it continued to impair performance up to 68 days post drug treatment. Evidence of behavioural tolerance was found as rats who had not experienced previous exposure to MDMA were more impaired when administered acute challenges of MDMA. Finally, as in the previous study the impairments produced by MDMA exposure, both from the binge regime and acute drug treatments, involved reference memory processes more than working memory processes.

Study 5: Binge and Repeated Acute Effects of MDMA on Radial Maze Acquisition

The findings from Study 3 and 4 of this thesis found evidence that binge MDMA exposure impairs learning in the partially baited radial arm maze. However, this impairment may be temporary in that by the end of training both MDMA treated and saline treated groups produce a similar level of performance. Recovery of neural impairments may underlie behavioural recovery as there is evidence subjects who are administered MDMA experience recovery of function. Indeed there is both behavioural (Brennan & Schenk, 2006) and neurochemical (Scanzello et al., 1993) evidence that the binge regime of MDMA used in these studies is accompanied by recovery over time. Therefore the current study examined what effect administering repeated acute doses of MDMA to subjects that had been pre-treated with a binge regime of MDMA would have on their ability to acquire the radial arm maze. Specifically this study investigated if subjects that continued to be exposed to MDMA would show recovery of the reference memory impairment seen in Studies 3 and 4 in the radial arm maze or whether subjects would remain impaired.

The majority of previous research that has examined the effects of binge MDMA exposure on cognition has utilised drug regimes that consist of large doses (10 to 20 mg/kg) given one to four times a day over a short period of several days. These types of regimes have been found to impair learning in the Morris water maze (Sprague et al., 2003; Able et al., 2005). Alternatively chronic regimes of MDMA (5 to 20 mg/kg) have been repeatedly administered for an extended period to young rats and performance on various cognitive tasks has been shown to be impaired when they were older (Vorhees et al., 2004; Skelton et al., 2006; Skelton et al., 2009; Broening et al., 2001; Williams et al., 2003).

In contrast there has been some research that has examined the effects of MDMA on cognition by administering long-term repeated acute doses of MDMA to subjects. For example Frederick et al. (1995) and Frederick and Paule (1997) administered ascending doses of MDMA (0.10 to 20 mg/kg) and found that long-term repeated exposure to the drug did not significantly alter performance on a previously learnt battery of cognitive tasks from baseline levels.

In addition there have been studies that have administered low acute doses of MDMA and then later conducted a chronic study by administering a high dose regime of MDMA to subjects. For example LeSage et al. (1993) administered a range of acute doses of MDMA (0.32 to 5.6 mg/kg) before administering a chronic regime of MDMA (3.2 to 5.6 mg/kg). The acute doses significantly impaired performance on a DMTS task; however chronic drug administration did not significantly alter behaviour compared to baseline performance. Also Byrne et al. (2000) initially administered rats an acute regime of MDMA (0.1 to 5.6 mg/kg) before exposing them to a binge regime (4 x 20 mg/kg for 4 days). Neither drug treatment had a significant effect on acquiring a DRL lever pressing task.

More recently, to try and represent human Ecstasy use more effectively, Skelton et al. (2008) compared different regimes of MDMA on acquiring the Cincinnati and Morris water maze. Rats were administered either 4 injections of 15 mg/kg administered once a day for a period of 4 weeks (4 lots of MDMA in total) or 4 injections of 15 mg/kg administered once in a single day (1 lot of MDMA in total). Skelton et al. (2008) found MDMA exposure produced deficits on both tasks. However, there were no differences in performance between the rats treated once with MDMA versus those treated repeatedly.

While there have been studies (Li et al., 1989; LeSage et al., 1993; Moyano et al., 2005) that have administered acute challenges of MDMA after binge exposure to the drug they have done so after the cognitive task being used has been acquired. As subjects have already learnt the task this dosing technique has usually been conducted to seek evidence of drug tolerance or sensitisation rather than investigating the effect of repeated MDMA exposure on learning processes per se.

Therefore to date there is no previous literature that has examined the effects of additional acute doses of MDMA on the acquisition of a task after subjects have already been exposed to a binge regime of the drug. Therefore, the current study adds to the previous MDMA literature by examining an issue not previously examined. In addition the repeated exposure to low doses of MDMA also extends the previous work of this thesis on whether behavioural tolerance or sensitisation develops following recurring MDMA exposure.

The Current Study

The current study utilised three groups of rats. The first group was the MDMA/MDMA group that were initially administered a binge regime of MDMA and then once week they were given one injection of an acute dose of MDMA (4.0 mg/kg). The second group was the Saline/MDMA group that was initially administered injections of saline and then once a week during training they were administered an acute dose of MDMA (4.0 mg/kg). The final group was the Saline/Saline control group that was initially administered injections of saline and during training they were given injections of saline once a week.

It was hypothesised that binge MDMA exposure would impair the acquisition of the radial arm maze compared to rats that were administered treatments of saline.

Additionally it was hypothesised that rats that were exposed to the acute MDMA treatments would show impaired performance compared to rats given acute treatments of saline. More specifically due to the previous findings from this thesis it was hypothesised that the impairments produced would be the result of reference memory errors rather than working memory errors.

Finally because this study design utilised repeated administrations of acute doses of MDMA it also enabled further examination of whether behavioural tolerance or sensitisation develops with repeated exposure to the drug. As a result of the findings from the Studies 3 and 4 that found evidence of behavioural tolerance it was hypothesised that the rats that were pre-treated with the binge regime of MDMA would be less impaired than the saline controls when administered subsequent acute doses of MDMA.

Method

Subjects

The subjects were twenty white male Sprague-Dawley rats that were approximately three to four months old at the beginning of the study. The rats were housed individually and were experimentally naïve at the beginning of the study. They were kept at approximately 85-90% (approximately 250 to 350 grams) of their free feeding body weight and began habituation training around one week of reaching this weight. They had continuous access to water and were kept on a 12:12-hour dark:light cycle and were run during the light phase of this cycle.

Apparatus/Materials

The maze and reinforcers used were the same as those stated in the general method section. Drugs used were saline 0.9 % and MDMA 10 mg/kg, which were prepared on the day of use by dissolving to the required dose in 0.9 % saline solution. During the tolerance phase of the experiment MDMA 4.0 mg/kg was used and it was also dissolved to the required dose in 0.9 % saline solution.

Procedure

The study used a mixed design utilising a between-subjects factor (binge MDMA versus saline pre-treatment) and a within subjects factor (acute MDMA versus acute saline) experimental design where the rats were divided into three groups. The first group was labelled the MDMA/MDMA group and at the beginning of the study prior to training its members received a binge regime (4 x 10mg/kg) of MDMA and then once a week throughout the study they received an acute dose (4 mg/kg) of MDMA. There were seven rats in this group. The second group, the Saline/MDMA group, was given saline at the beginning of the study and thereafter was administered an acute dose (4 mg/kg) of MDMA once a week. There were seven rats in this group. Finally the third group was designated the Saline/Saline group which, at the beginning of the study received saline and once a week was given subsequent acute injections of saline. This group acted as the control group and there were six rats in this group.

The initial study design consisted of four groups as it involved a MDMA/Saline treatment group. However, for practical and ethical reasons the number of groups was reduced as the current design used fewer rats. In addition the results from the group of rats that were administered a binge regime of MDMA in Study 4 could be used as a comparison for the performance of the MDMA/MDMA group.

Each rat was assigned a set of four reinforced arms and four non-reinforced arms within the maze. The three groups of rats had the same sets of reinforced and non-reinforced arms, so that the first rat in all groups had the same reinforced arms and so on. This was done to control for difficulty of the task in case some sets of maze arms were easier to learn that others. Training was conducted in the same way as outlined in the general method section. However, in this study the rats received the habituation pre-training before binge drug treatments were administered. The day after completing the habituation phase, rats were given four injections of either 10 mg/kg of MDMA or 0.9 % of saline. The following day rats were given a rest day where they were not run in the maze.

Assigned reinforcer arm training began the after the rest day (two days after the injections). Rats were run within their matched groups as this was more convenient in terms of not having to change reinforcer arms so often. However, to control for order or odour scenting in the maze the running was counterbalanced so that which rat ran first was changed on every third day so that each rat was not able to solve the task simply by following the scent of the other rats. To examine whether repeated MDMA exposure would affect performance each group of rats received an acute injection of either MDMA (4.0 mg/kg) or saline (0.9%) once a week. These injections were administered via an i.p. injection 20 minutes before they were run in the maze.

Results

Training Data

In all figures error bars show standard error of the mean. Daily percent correct figures were calculated by averaging across the three trials conducted each day to

obtain an average for the session for each rat. Group values were then obtained for each of the three groups by averaging the session performance across all rats in group. These data are presented below in Figure 25 and show that the Saline/Saline control group started the task at around an average of 45% which is slightly above chance for this paradigm (33% chance level) and continue on at a relatively steady rate until sessions 17-18 when they start to plateau at around ninety percent. Therefore, this group of rats learnt the task quite quickly achieving a high level of accuracy with not a lot of variability. It is also clear from the figure below that on the acute drug administration days which occurred during sessions 8, 15, 22, 29, 36 and 43, the Saline/Saline control group received acute doses of saline and there was no noticeable change in their performance.

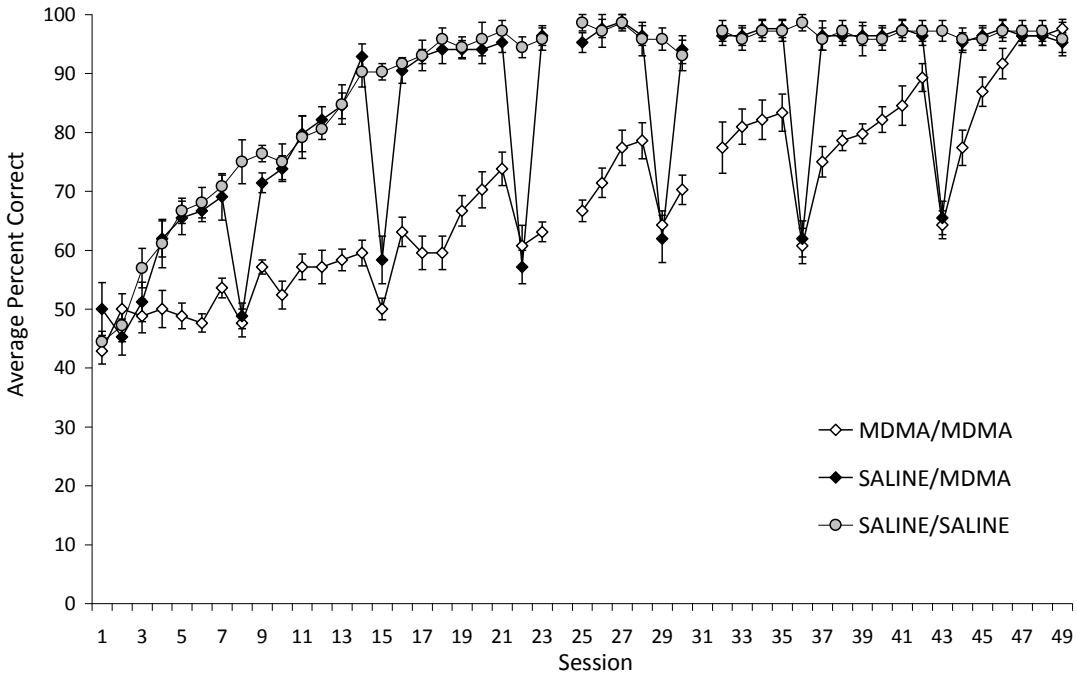

Figure 25: Average Percent Correct for the three rat groups and the effect of the acute drug treatment given once a week on training session 8, 15, 22, 29, 36 and 43.

The Saline/MDMA group performed similarly to the Saline/Saline control group at the beginning of the study and their data almost directly maps on that of the Saline/Saline groups. However when the Saline/MDMA group were administered acute doses of MDMA their accuracy dramatically dropped indicating that the drug impaired their performance**.** However, what is interesting is that their performance the day after receiving the MDMA does not appear to be affected suggesting that the acute doses of MDMA produced very short-term effects on performance.

Finally if we examine the MDMA/MDMA group it is clear that they are slower to learn the task compared to both control groups. They start off at a similar level but soon after this group's accuracy is well below that of the other two groups and continues to increase at a much slower rate. However, by the end of training they do produce a similar level of performance compared to the two saline control groups. During the acute drug days this group also produced a large drop in performance and there appears to be some evidence of this impairment affecting performance the day after the acute drug session.

To analyse the accuracy of the three groups during training the data for the acute drug administration days was removed and a 2-way mixed ANOVA was conducted. The analysis revealed a significant interaction between training and group, $F(80, 680) = 6.67$, $p < 0.05$ ($p = 0.00$) and main effects for training, $F(40, 80) = 117.20$, $p < 0.05$ ($p = 0.00$) and group, $F(1, 17) = 211.41$, $p < 0.05$ ($p = 0.00$). From Figure 25 it is clear that while the three groups initially perform at the same level of performance the MDMA/MDMA group was then significantly worse than the two saline control groups for majority of the remaining sessions. However all groups improved over the training sessions with the MDMA/MDMA group improving at a slower rate than the two control groups.

Average trial completion times were also calculated for the three groups and are depicted in Figure 26. This figure shows that all three groups became quicker at completing trials over the first sessions of training. Overall the Saline/Saline control group seems to improve slightly faster compared to the two other groups. Also there is no obvious effect on performance during the acute drug administration sessions for this group. Again performance of the Saline/MDMA group is similar to that of the-Saline/Saline group, except for a brief period at the beginning of training and during the acute drug administration sessions where their trial completion times dramatically increase. Finally the MDMA/MDMA group again initially produce a similar level of performance to that of the two control groups but approximately half way through the training sessions they began to produce slightly slower trial completion times. In addition during the acute drug administration sessions their performance also becomes impaired as indicated by the large increase in trial completion times.

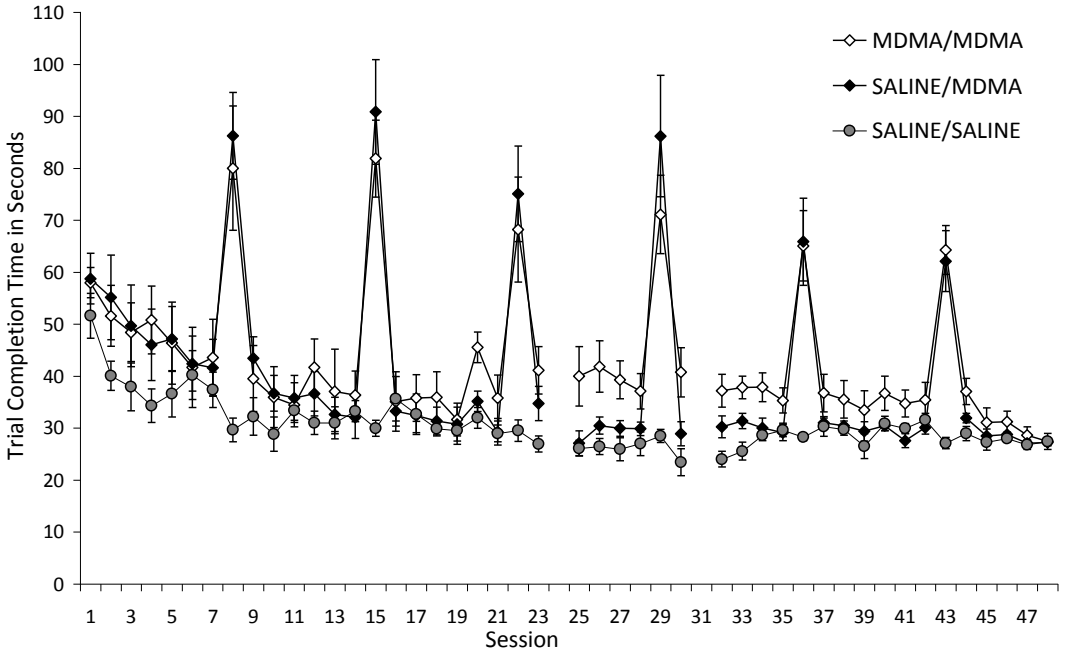

Figure 26: Average trial completion time for the three rat groups and the effect of the acute drug treatment given once a week on training session 8, 15, 22, 29, 36 and 43.

Trial completion time data for the three groups across the training sessions was analysed by removing the data from the acute drug administration sessions. A 2-way mixed ANOVA revealed a significant interaction between training and group, $F(80, 680) = 1.44$, $p < 0.05$ ($p = 0.01$) and a main effect for training, $F(40, 80) = 16.16$ $p < 0.05$ ($p = 0.00$). There was no main effect for group, $F(1, 17) = 1.79$, $p > 0.05$ ($p = 0.20$).

Working Vs Reference Memory Data

To examine the types of error made within the radial maze average error percentages were calculated for the three groups (using the same procedure as previous studies). The average percent of working memory errors made by the three groups during training are depicted in Figure 27. It clearly shows that few working memory errors were made by any of the three groups and that the number of errors did not seem to change dramatically over the training period. While there appears to be a very slight increase in working memory errors by the MDMA/MDMA group half way through the training sessions there does not seem to be a clear overall pattern. Also there is no obvious effect of acute drug treatment session seen on the number of working memory errors made by any of the three groups.

To analyse the working memory data for the three groups during training the data from the acute drug sessions was removed. A 2-way mixed ANOVA found a significant interaction between training and group, $F(80, 680) = 1.68$, $p < 0.05$ ($p = 0.00$). However no main effect of training was found, $F(40, 80) = 1.11$, $p > 0.05$ ($p = 0.31$) and no main effect of group, $F(1, 17) = 3.47$, $p > 0.05$ ($p = 0.05$).

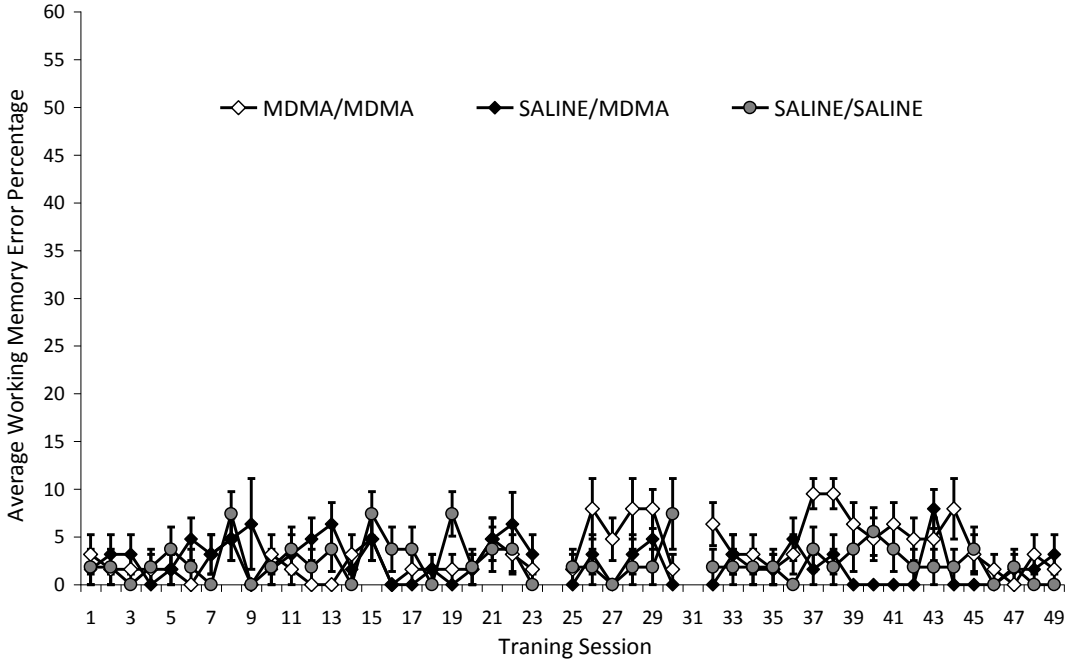

Figure 27: Average working memory errors for the three rat groups and the effect of the acute drug treatment given once a week on training session 8, 15, 22, 29, 36 and 43.

The data for the average reference memory percentage errors for the three groups during training data is shown in Figure 28. This figure clearly shows that during training more reference memory errors were made than working memory errors. At the beginning of training all three groups made a large number of reference memory errors. During the early training sessions the number of reference memory errors rapidly decreases in the two saline control groups. Therefore it appears the Saline/MDMA and Saline/Saline groups show a faster rate of learning than the MDMA/MDMA group. Gradually the number of reference memory errors decreases for all groups as they acquire the task and eventually the MDMA/MDMA group are able to achieve a similar level of performance to the control groups.

In Figure 28 the effects of the acute drug sessions are also quite evident. The Saline/MDMA group and the MDMA/MDMA groups both make more reference memory errors when administered acute doses of MDMA. While the number of

reference memory errors made between the MDMA/MDMA and the Saline/Saline groups appears similar it should be pointed out that the Saline/MDMA group's performance is more impaired in terms of how many reference memory errors they made during acute drug treatment in relation to how many they made the day before.

Again the interesting finding that the MDMA/MDMA group are more affected than the Saline/MDMA group on the days following acute drug treatment is evident in terms of the number of reference memory errors made in the sessions immediately after drug exposure days. The Saline/MDMA group are quite impaired on the day of acute drug administration as evidenced by a large increase in the number of reference memory errors made. However, this deficit is short lived with performance returning to baseline levels the day immediately after acute drug administration. In contrast, although MDMA/MDMA group also show an increase in the number of reference memory error made during the drug administration sessions their performance does not immediately return to baseline levels but instead remains slightly impaired on the days following the acute injections of MDMA.

To analyse the reference memory data for the three groups during training the data from the acute drug sessions was removed and a 2-way mixed ANOVA was conducted. Significant main effects for training, $F(40, 80) = 125.57$, $p < 0.05$ ($p = 0.00$) and group, $F(1, 17) = 198.61$, $p < 0.05$ ($p = 0.00$) were found but these were moderated by a significant interaction between training and group, $F(80, 680) = 7.70$, $p < 0.05$ ($p = 0.00$). From Figure 28 it is clear that while initially all three groups produced a similar number of reference memory errors the MDMA/MDMA group produced significantly more reference memory errors than the two saline control groups for the majority of the remaining sessions. However all groups improved over the sessions with the MDMA/MDMA group improving at a slower rate than the other two groups.

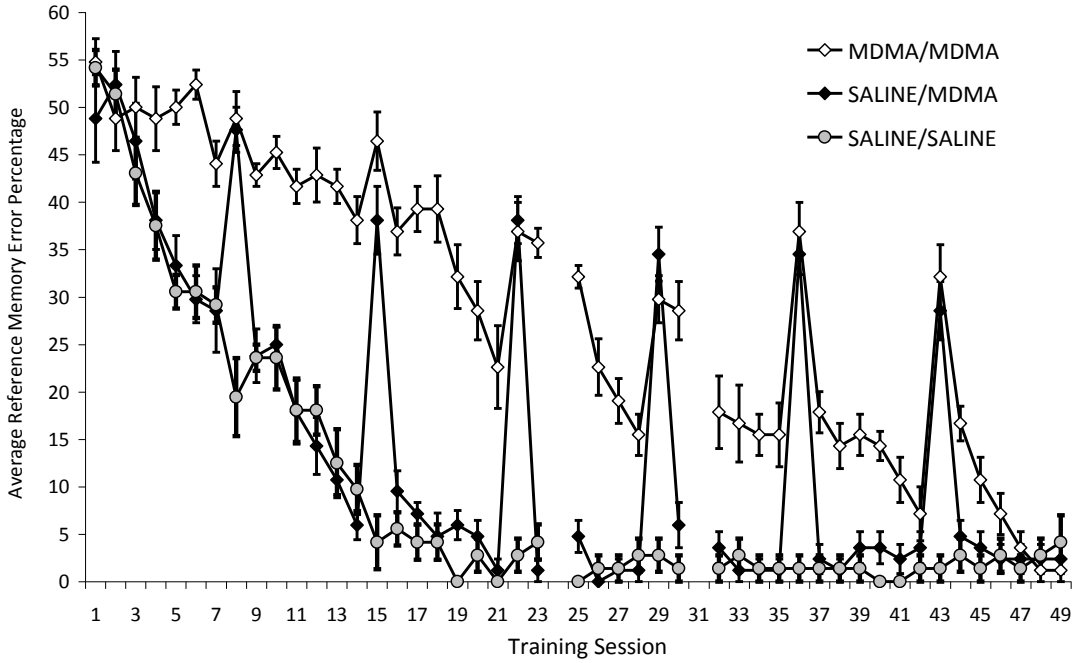

Figure 28: Average reference memory errors for the three rat groups and the effect of the acute drug treatment given once a week on training session 8, 15, 22, 29, 36 and 43.

In conclusion repeated MDMA administration impaired acquisition of the radial arm maze in the group of rats that were pre-treated with a binge regime of MDMA. In addition all groups made more reference memory errors than working memory errors during task acquisition; however the MDMA/MDMA made significantly more than the other groups. Replicating the findings from the first two studies of the current thesis it was found that acute MDMA administration impaired accuracy, increased trial completion times and produced more reference memory errors than working memory errors.

Acute Drug Treatment Percent Correct Data

To examine the effects of the acute drug treatments and to find further evidence of behavioural tolerance or sensitisation the average relative change in performance was calculated. This involved dividing performance on the day of acute drug treatment by the performance of the day before (baseline). This was conducted to provide an indication of the relative change between what subjects were like before and during the drug sessions. This analysis enabled the subjects starting point to be taken into account when examining the impairment produced by drug exposure. If the average relative change was a value larger than one it meant the rats performance improved on the drug day compared to the day before. Finally if the value was less than one it meant the subjects were impaired and the smaller this value the greater the impairment. These data are presented in Figure 29.

The Saline/Saline controls produced values of around one and above as administering saline did not alter performance. The Saline/MDMA group's performance changed dramatically when administered MDMA. Finally it is evident that performance of the MDMA/MDMA group did not drop dramatically during the first drug treatments but their performance gradually got worse as the acute drug sessions continued.

When comparing the performance of the MDMA/MDMA to the Saline/MDMA groups it would appear that initially there may be evidence of behavioural tolerance as the Saline/MDMA group's performance shows more of a dramatic decrease. Therefore during the first couple of sessions it would appear that rats that had previously been exposed to MDMA were less impaired when administered subsequent acute doses of the drug. However, by the end of the study this pattern dissipates. So there maybe some initial evidence of behavioural tolerance that

progressively declines as the performance of the MDMA/MDMA group becomes gradually more impaired and hence becoming similar to that of the Saline/MDMA group.

Possible explanations for this effect may be due to continued training, repeated exposure to acute MDMA or simply as a product of recovery of function at a neuro-chemical level. However a more likely interpretation is that the initial evidence for behavioural tolerance during the first few trials is produced by a floor effect. This is due to the fact that the MDMA/MDMA group is performing at such a low level of accuracy that when the acute doses of MDMA are administered performance does not decrease dramatically because performance cannot show any further impairment. Hence in the current study there is no convincing evidence of behavioural tolerance.

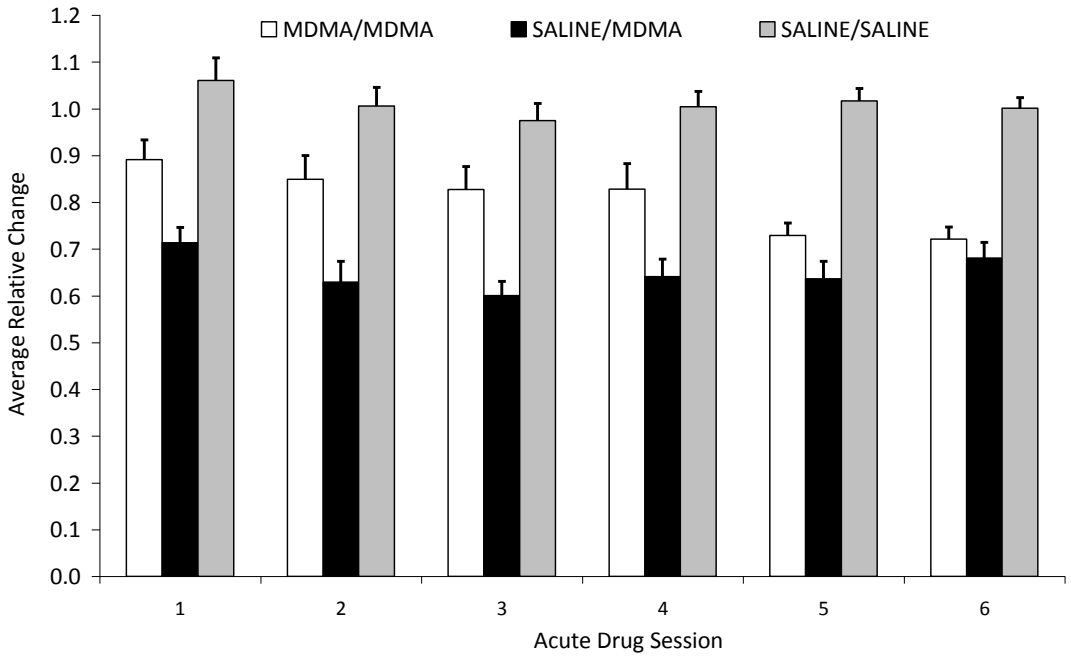

Figure 29: Average relative change from the day before acute drug treatment and the day of acute drug treatment.

A 3-way mixed ANOVA was used to analyse the percent correct data from the day before drug treatment with performance on the day of acute drug treatment across all six drug sessions and between the three groups. A significant 3-way interaction was found, $F(10, 85) = 2.11$, $p < 0.05$ ($p = 0.03$). Therefore there was a significant difference in accuracy for the day before drug treatment versus the day of drug administration and this changed with repeated drug exposure and depended on group. Clearly when examining Figure 25 in the previous section, this effect is driven by both the MDMA/MDMA group and the Saline/MDMA group whose performance significantly decreases during the day of drug treatment compared to the Saline/Saline control group and the fact that this difference gets larger as subjects acquire the task and their performance the day before treatment improves hence producing a larger drop in performance when MDMA is administered.

In addition a 3-way mixed ANOVA was also used to analyse the data from the day after drug treatment with performance on the day of acute drug treatment across all six drug sessions and between the three different groups. An interaction was found, $F(10, 85) = 2.06$, $p < 0.05$ ($p = 0.04$), indicating a significant difference in accuracy between performance on the day of drug treatments versus the day after treatment which depended on repeated drug exposure and drug treatment on group. Again when examining Figure 25 it can be seen that this effect is driven by the MDMA/MDMA group and the Saline/MDMA group whose performance differs from the day of acute drug exposure and the day after. While the Saline/MDMA group's performance is impaired on drug exposure days performance on the day after drug exposure resumes to baseline levels. However, the MDMA/MDMA group is impaired on drug days but also appears to remain impaired to a degree on the day after drug exposure as performance does not return to baseline levels.

To further examine these effects the average effect of acute drug treatment was calculated by collapsing across the six drug treatment sessions. This data is presented in Figure 30 and shows that the MDMA/MDMA group produced an overall lower level of accuracy compared to the two other groups. It is also evident that performance during MDMA administration produced a similar level of performance for both the MDMA/MDMA and the Saline/MDMA groups. This figure also shows that during the day after drug treatment there is a residual drug effect for the MDMA/MDMA where performance remains impaired. In contrast the Saline/MDMA group's performance does not show any residual effect with performance returning to levels similar to that seen the day before drug treatment. In addition it is clear that for the Saline/Saline group had a very high level of performance and during the drug administration days where this group received saline their performance did not change.

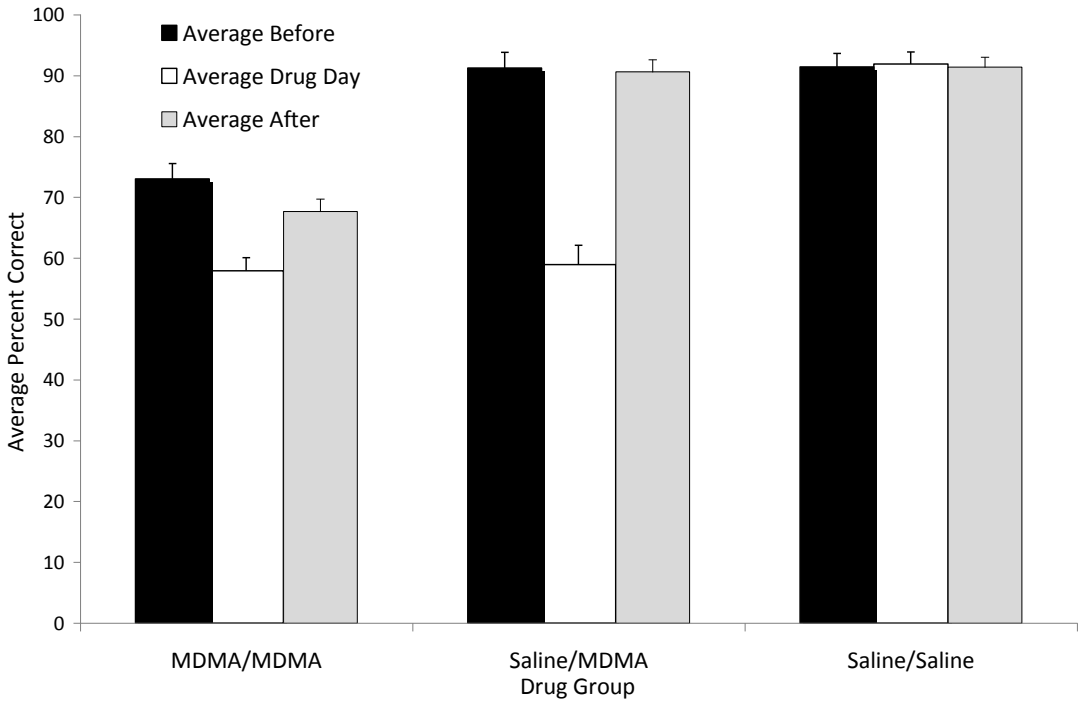

Figure 30: Average percent correct for the day before, the day of drug treatment and the day after treatment for the three drug groups collapsed across the six acute drug sessions.

A one-way repeated measures ANOVA was conducted for each of the three drug treatment groups comparing performance on the day before, the day of and the day after acute drug administration. For the Saline/Saline group no effect was found, $F(2, 10) = 0.10$, $p > 0.05$ ($p = 0.91$), indicating there was no significant difference in performance between the day before, the day of or the day after acute drug treatment.

For the Saline/MDMA group there was an effect of day, $F(2, 12) = 279.53$, $p < 0.05$ ($p = 0.00$), suggesting a difference in accuracy between the day before, the day of and the day after drug administration. Paired sample t-tests were conducted to examine where this difference occurred. Rats in this group were significantly less accurate on the day of acute drug treatment compared to the day before ($t(6)=16.93$, $p < 0.05$) ($p = 0.00$). Subjects were also significantly less accurate on the day of drug exposure compared to the day after ($t(6)=-27.58$, $p < 0.05$) ($p = 0.00$). Finally there was no significant difference between performance on the day before and the day after drug treatment ($t(6)=0.39$, $p > 0.05$) ($p = 0.71$). Therefore the Saline/MDMA group's performance returned to baseline levels the day after drug exposure and no residual drug effects were evident.

Finally when analysing the performance of the MDMA/MDMA group an effect of day was produced, $F(2, 12) = 49.66$, $p < 0.05$ ($p = 0.00$). Therefore there was a significant difference in accuracy between the day before, the day of and the day after acute drug exposure. Subjects were significantly less accurate on the days they were given acute doses of MDMA compared to the day before ($t(6) = 9.87$, $p < 0.05$) ($p = 0.00$). Again subjects were significantly less accurate on the days they were administered acute MDMA compared to the day after ($t(6) = -6.06$, $p < 0.05$) ($p = 0.00$). Finally rats in the MDMA/MDMA group were significantly less accurate the day after acute MDMA administration compared to the day before drug treatment ($t(6) = -3.65$, $p < 0.05$) ($p = 0.01$). This suggests that this group experienced a significant

residual drug effect where the acute doses of MDMA continued to impair performance the day after drug exposure.

Acute Reference Memory Data

A 3-way mixed ANOVA was used to analyse the day before versus the day of drug treatment for reference memory errors. There was no significant interaction between repeated acute drug exposure with performance the day before versus the day of acute drug treatment and group, $F(10, 85) = 1.86$, $p > 0.05$ ($p = 0.06$). Therefore there were no significant differences in the number of reference memory errors made with repeated administrations of MDMA and saline during the day before treatments versus the day of drug treatments and between the three drug treatment groups. However, there was an interaction between the performance on the day before acute drug treatment versus the day of acute drug treatment with group, $F(2, 17) = 144.85$, $p < 0.05$ ($p = 0.00$). Therefore there was a significant difference between the three drug treatment groups in the number of reference memory errors made the day before versus the day of drug treatment.

The reference memory data for the day after drug treatment versus the day of drug treatment was also analysed using a 3-way repeated measures ANOVA and found there was a significant interaction between repeated acute drug exposure with performance the day after versus the day of acute drug treatment and group, $F(10, 85) = 3.03$, $p < 0.05$ ($p = 0.00$). Therefore there was a significant difference in the number of reference memory errors made with repeated administrations of MDMA and saline during the day after treatments versus the day of drug treatments and between the three drug treatment groups. In addition there was an interaction between the performance

on the day after acute drug treatment versus the day of acute drug treatment with group, $F(2, 17) = 123.48$, $p < 0.05$ ($p = 0.00$). Hence there was a significant difference between the three drug treatment groups in the number of reference memory errors made the day after versus the day of drug treatment.

Therefore to further examine this trend the average effect of acute drug treatment was calculated by collapsing across the six drug treatment sessions. This data is presented in Figure 31 and shows that overall the MDMA/MDMA group produced more reference memory errors than the other two groups. It is also clear that for the day before and the day after there is no obvious difference in the number of reference memory errors produced by the MDMA/MDMA and the Saline/MDMA groups. However on the day of drug exposure both the Saline/MDMA and the MDMA/MDMA groups produce a large number of reference memory errors clearly indicating impairment with acute drug exposure.

In addition Figure 31 shows there is some evidence of a residual drug effect for the MDMA/MDMA where the number of reference memory errors does not return to baseline levels on the day after drug administration. In contrast the Saline/MDMA group's performance does not show any residual effect with performance returning to baseline levels. It is also evident that the Saline/Saline group produced very few reference memory errors overall and on the drug administration days where this group received saline their performance was not noticeably altered.

A one-way repeated measures ANOVA was conducted for each of the three drug treatment groups comparing the amount of reference memory errors on the day before, the day of and the day after acute drug administration. For the Saline/Saline group no effect was found, $F(2, 10) = 2.44$, $p > 0.05$ ($p = 0.14$), indicating there was

no significant difference in performance between the day before, the day of or the day after acute drug treatment.

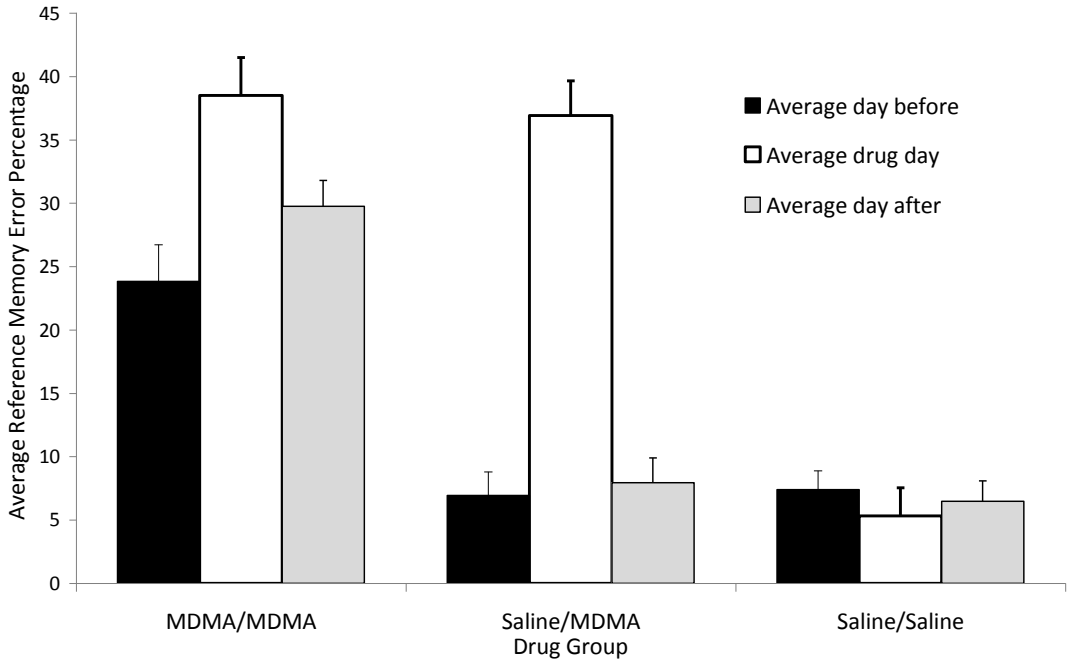

Figure 31: Average reference memory error percentage for the day before, the day of drug treatment and the day after treatment for the three drug groups collapsed across the six acute drug sessions.

For the Saline/MDMA group there was an effect of day, $F(2, 12) = 822.52$, $p < 0.05$ ($p = 0.00$), suggesting a difference in the number of reference memory errors between the day before, the day of and the day after drug administration. Therefore paired sample t-tests were conducted to examine where this difference occurred. Rats in this group produced significantly more reference memory errors on the day of acute drug treatment compared to the day before ($t(6) = -30.00$, $p < 0.05$) ($p = 0.00$). Subjects also produced significantly more reference memory errors on the day of drug exposure compared to the day after ($t(6) = -45.418$, $p < 0.05$) ($p = 0.00$). However

there was no significant difference in the amount of reference memory errors on the day before and the day after drug treatment ($t(6) = -1.18$, $p > 0.05$) ($p = 0.28$). Therefore the Saline/MDMA group's performance returned to baseline levels the day after drug exposure and no residual drug effects were produced.

When analysing the reference memory data for the MDMA/MDMA group an effect of day was also found, $F(2, 12) = 47.32$, $p < 0.05$ ($p = 0.00$). Hence there was a significant difference in the number of reference memory errors made between the day before, the day of and the day after acute drug exposure. Subjects produced significantly more reference memory errors on the day of drug treatment compared to the day before ($t(6) = -8.72$, $p < 0.05$) ($p = 0.00$). Also subjects produced significantly more reference memory errors on the days they were administered acute MDMA compared to the day after ($t(6) = 4.57$, $p < 0.05$) ($p = 0.00$). In addition the rats in the MDMA/MDMA group produced significantly more reference memory errors the day after acute MDMA administration compared to the day before drug treatment ($t(6) = -9.05$, $p < 0.05$) ($p = 0.00$). Therefore the MDMA/MDMA group produced evidence of a residual drug effect where acute doses of MDMA continued to impair performance the day after drug exposure.

Acute Working Memory Data

A 3-way mixed ANOVA was used to analyse the working memory data from the day before drug treatment with performance on the day of acute drug treatment across all six drug sessions and between the three groups. No significant interaction was found between repeated acute drug exposure, performance the day before versus the day of acute drug treatment and group, $F(10, 85) = 0.80$, $p > 0.05$ ($p = 0.63$).

Therefore there were no significant differences in the number of working memory errors made with repeated administrations of MDMA and saline during the day before treatments versus the day of drug treatments and between the three drug treatment groups.

However, a 3-way mixed ANOVA was also used to analyse the working memory. There was a significant interaction between repeated acute drug exposure with performance the day after versus the day of acute drug treatment and group, $F(10, 85) = 2.27$, $p < 0.05$ ($p = 0.02$). Therefore there was a significant difference in the number of working memory errors made that was dependent on repeated drug exposure, performance on the day after versus the day of acute drug treatment and chronic drug treatment group.

However it is obvious from the lack of a clear pattern in Figure 29 that there is no consistent change in the amount of working memory errors made across the training sessions for any group. This is supported by the non-significant 3-way interaction between day before versus day of drug treatment, repeated drug exposure and group. The only exception to support that lack of an effect for working memory was the 3-way interaction found between day after versus day of drug treatment, repeated drug exposure and group which just reached significance.

Acute Trial Completion Time Data

There was no interaction between repeated acute drug exposure with performance the day before versus the day of acute drug treatment and group, $F(10, 85) = 0.80$, $p > 0.05$ ($p = 0.63$). Therefore there were no significant differences in the average amount of time to complete trials with repeated drug administration, the

difference in performance compared to the day before and the day of drug administration and drug group as can be seen in Figure 26.

In addition there was no interaction between repeated acute drug exposure with performance the day after versus the day of acute drug treatment and group, $F(10, 85) = 1.37$, $p > 0.05$ ($p = 0.21$). Therefore there were no significant differences in the average amount of time to complete trials with repeated drug administration, the difference in performance compared to the day after and the day of drug administration and drug treatment group.

Discussion

To reiterate, the aim of the current study was to examine the effects of repeated acute MDMA exposure on acquisition of the partially baited radial maze. It was found that acute administration of MDMA following binge MDMA exposure impaired task acquisition. Hence the hypothesis that the binge regime of MDMA would significantly impair acquisition of the radial maze was supported. In addition when comparing the findings from this study with those of Study 4, that utilised the same chronic regime of MDMA and the same gap between drug exposure and the commencement of training as the current study, it was found that the subsequent MDMA exposure may have additionally impaired task acquisition. In the current study it took 47 sessions for the group that received binge MDMA treatment to perform at a similar level of performance to the groups that were administered saline regimes. However, in Study 4 this took only 22 sessions to occur. Therefore it would appear receiving a binge regime of MDMA and then receiving multiple acute doses of the drug impairs learning processes more so than receiving the binge MDMA regime alone.

Also consistent with our hypothesis, acute administration of MDMA also significantly impaired accuracy. Of interest it seems that administering acute doses of MDMA to the pre-treated binge rats produced longer lasting effects on performance. The rats that were initially pre-treated with saline and then administered acute doses of MDMA were impaired on the day of drug exposure but by the following day their performance recovered to baseline levels. However for the rats that were pre-treated with a binge regime of MDMA and then given acute doses of MDMA once a week their performance was impaired during the drug testing session. However in addition there were residual effects whereby their performance remained significantly impaired the day after acute exposure.

During acute drug treatment sessions there was some initial indication of behavioural tolerance in that the saline control rats at first appeared more disrupted by the acute doses of MDMA than the binge MDMA treated rats. However this effect seemed to dissipate as training continued suggesting it may have been due to a floor effect whereby the binge MDMA treated rats could not show such a large degree of impairment initially as their performance was so poor. Therefore contrary to the findings from Study 3 and 4 the current study did not find compelling evidence of behavioural tolerance with repeated MDMA exposure hence our hypothesis that behavioural tolerance would occur was not supported.

Binge MDMA exposure appeared to have little effect on the amount of time it took rats to complete trials. There were no significant differences found between the rats that received the binge regime of MDMA and those that received saline during acquisition of the task. Therefore it is unlikely that the impairments found in the MDMA/MDMA group were not due to motor impairments caused by binge exposure to MDMA. However, performance during acute MDMA administration produced noticeable increases in trial completion time which is consistent with the findings from

Study 1 and 2 of the current thesis. However, it should be noted that with acute MDMA exposure there were no obvious differences in trial completion time between the group of rats that were administered the binge regime of MDMA and those given saline.

Binge MDMA exposure also did not appear to obviously impair working memory processes and on the whole very few working memory errors were made by any group. In addition acute MDMA exposure did not produce a marked increase in working memory errors in any group. Therefore it seems that the impairments found in this study were not due to an MDMA induced deficit in working memory processes. Therefore the deficits produced by MDMA exposure were primarily the result of a reference memory impairment. Whereby during acquisition the group that were administered the binge regime of MDMA produced significantly more reference memory errors than those administered saline. In addition during acute MDMA exposure more reference memory errors were made than working memory errors. Therefore supporting our hypothesis it was found that both binge and acute administration of MDMA produced deficits in reference memory processes more so than working memory processes. Again this finding concurs with the previous studies of the current thesis.

While this study failed to find persuasive evidence of behavioural tolerance it should be pointed out that there are differences between the current and previous studies of this thesis. In Studies 3 and 4 behavioural tolerance was assessed by administering the acute doses of MDMA after the task had been acquired. As behavioural tolerance was found it suggests that previous binge MDMA exposure produced a protective factor in that performance was less impaired than those who had not received the previous MDMA exposure when acute doses of MDMA were administered. However in the current study the acute doses of MDMA were

administered while learning of the task was still occurring. Therefore it would appear that acute administration of MDMA during task acquisition is more disruptive while learning is still taking place. Therefore this may indicate that learning processes are more disrupted by MDMA exposure compared to memory processes that are used once the task has been acquired.

In addition it may be that administering repeated acute doses of MDMA may disrupt the development of behavioural tolerance. It has been argued that repeated low doses of acute MDMA results in behavioural sensitisation while large doses of MDMA results in the development of tolerance (Brennan & Schenk, 2006). Therefore as this study utilised both it may have inhibited the development of behavioural tolerance with sensitisation processes from the acute doses of MDMA and tolerance processes from the binge MDMA exposure effectively cancelling each other out. Future research could examine this phenomenon further by manipulating the exposure of MDMA in different doses and regimes to examine which conditions produce behavioural tolerance or sensitisation.

Due to the novelty in design of the current study the findings are difficult to compare with previous research as no studies to date have administered repeated acute doses of MDMA after initial binge exposure while subjects are still learning a cognitive task. Thus the findings of the current study add something new to the existing literature on the effects of MDMA on cognition. In particular it would appear that additional exposure to MDMA, while still acquiring a cognitive task, seems to impair learning. This is a novel finding as previous research tends to administer large regimes of MDMA before training and if subsequent MDMA exposure occurs it does so after the task is acquired. Thus while previous work has been able to examine whether drug tolerance or sensitisation occurs it has not been able to examine what effect repeated MDMA exposure has on actual learning processes during task

acquisition. In addition the current study adds to the previous literature as it was able to concurrently assess working and reference memory processes. It was found that reference memory processes in particular seem to be impaired while working memory processes seemed largely unaffected which augments previous work (Broening et al., 2001; Williams et al., 2003; Sprague et al., 2003; Vorhees, 2004; Skelton et al., 2006; Able et al., 2006; Skelton et al., 2008; Skelton et al., 2009) using Morris water maze tasks that have had to assess these processes separately.

In conclusion it was found that rats that were administered a binge regime of MDMA and then given acute doses of MDMA once a week were significantly impaired at acquiring the radial arm maze task compared to rats that did not receive the regime of MDMA. Also this impairment involved reference memory processes as opposed to working memory processes and did not seem to affect trial completion time. Repeated acute administration of MDMA did not result in behavioural tolerance due to a potential floor effect. Also the MDMA/MDMA group experienced a significant residual drug effect from the acute doses of MDMA as they remained impaired the day after drug administration. Therefore this study suggests that learning processes may be more disrupted by repeated MDMA exposure than memory processes. This study indicates that repeated MDMA exposure may inhibit the recovery of function seen with binge MDMA exposure in Study 4 as subjects took longer to acquire the task when administered additional acute injections of MDMA.

Binge MDMA Discussion

Recap of Main Findings

In Study 3 a binge regime of MDMA (4 x 10 mg/kg) was administered to rats and eight to ten weeks later their ability to acquire the partially baited radial arm maze was compared against saline controls. No significant difference on any measure of performance was found between the two groups ability to learn the task. To examine whether further drug exposure would have any effect on performance once the task has been acquired, another binge regime of MDMA was administered to the previously treated MDMA rats. This resulted in a significant but transient impairment in performance. In the last phase of the study an acute dose of MDMA and saline were administered to examine whether behavioural tolerance or sensitisation would occur. Evidence of behavioural tolerance was found as the binge MDMA treated rats were significantly less impaired when administered acute MDMA than the saline controls.

The next study examined whether the lack of an effect produced by the initial exposure to MDMA in Study 3 was due to the time period between drug administration and training or due to the amount of MDMA administered. Therefore in Study 4 the previously used binge regime of MDMA was administered to rats three days before training began in the maze. The MDMA treated group were significantly slower in acquiring the task compared to the saline controls. However, the MDMA treated rats were able to eventually perform at a similar level to the controls suggesting MDMA exposure impaired their rate of learning but did not prevent them from acquiring the task. The rules of the task were then reversed to examine the permanence of the cognitive impairment and to investigate the effect of MDMA treatment on the

ability to adapt behaviour to changing consequences. The MDMA treated rats took significantly longer to learn the new task compared to saline controls. Therefore binge MDMA exposure significantly impaired the ability of subjects to alter their behaviour when faced with a change in task demands. By the end of training they were able to perform at a similar level to the control group showing they could eventually learn to alter their behaviour. The finding that MDMA treated rats that appeared to have recovered from the effects of the drug in the first phase of the study went on to show impairment when the rules of the task were reversed indicates that the learning impairments produced by MDMA may be long-term in nature. This study also examined the effects of acute administration of MDMA and saline on rats that had previously been treated with a binge MDMA regime of saline. As in the previous study evidence of behavioural tolerance was found.

The final study of the current thesis examined the effects of repeated MDMA exposure on task acquisition in the partially baited radial maze. Rats that were exposed to a binge regime of MDMA and then administered repeated acute doses of MDMA were impaired in acquiring the radial arm maze compared to rats that were pre-treated with saline. In addition administering repeated acute doses of MDMA to rats that had already received a binge regime of MDMA further impaired their ability to acquire the task taking approximately 47 sessions to perform at a similar level to saline controls. This is more than double the number of training sessions compared to the results from Study 4 that used the same task, breed of rat and regime of MDMA. Performance was significantly impaired during sessions that acute doses of MDMA were administered. However there was no convincing evidence of behavioural tolerance with repeated MDMA administration. Of interest the group that received the binge regime of MDMA were impaired on the day of acute MDMA exposure and the day after whereas saline controls were not. Thus this group produced evidence of a residual drug effect.

In conclusion the findings from Studies 3, 4 and 5 that examined the effect of binge MDMA exposure on cognitive performance all produced some form of an impairment in accuracy in the partially baited radial maze. In addition in all three studies these impairments were the result of MDMA exposure producing more reference memory errors than working memory errors. This suggests that binge MDMA administration affects reference memory processes more so than working memory processes. Binge MDMA exposure generally failed to produce an effect on trial completion time. Thus the impairments found with drug exposure cannot be explained in terms of motor impairments caused by drug exposure. Also supporting the findings from Study 1 and 2 in the first half of this thesis it was found that acute MDMA exposure impaired accuracy in the radial arm maze as well as increasing trial completion times. Acute MDMA exposure produced more reference memory errors than working memory errors.

Comparison to Previous Chronic/Binge MDMA Literature

Unlike the findings of the current thesis most studies (LeSage et al., 1993; Taffe et al., 2001; Frederick et al., 1995; Frederick & Paule, 1997) that have examined the effects of chronic and binge MDMA exposure on cognition by utilising DMTS and DNMTS tasks have failed to show that the drug significantly affects performance from baseline levels. However it should be noted that in these studies the cognitive task had already been acquired and while performance was affected during drug administration there were no long-term deficits. Hence chronic or binge MDMA exposure does not seem to affect performance on an already acquired task. In addition the results from this thesis indicate that reference memory processes are more affected by binge MDMA exposure than working memory processes. While DMTS and DNMTS tasks

involve a reference memory component they are primarily used to assess working memory processes (Harper et al., 2005). Therefore these tasks may not involve a large enough reference memory component to produce impairment following chronic or binge MDMA administration.

In contrast to the findings of the current thesis Winsauer et al. (2002) also failed to find evidence of binge MDMA exposure impairing performance on a cognitive task. A repeated acquisition task was used where each session subjects had to learn a sequence of lever presses to gain reinforcement. While this task is used to assess learning as the lever sequences change each session, subjects were pre-trained before drug exposure. Hence they had acquired the general rules of the task before MDMA was administered. Therefore a possible reason why Winsauer et al. (2002) failed to find an effect with binge MDMA exposure may be similar to the DMTS task findings whereby as subjects have had experience with the task their performance is harder to disrupt with drug exposure compared to tasks that subjects are still learning.

Similarly Moyano et al. (2005) found that a binge regime of MDMA did not significantly alter performance from controls on a passive avoidance task when tested seven days after drug exposure. However, when later challenged with an acute dose of MDMA the previously MDMA treated animals were significantly impaired compared to controls. Therefore although the initial regime of MDMA did not appear to impair performance the finding that these rats were later more sensitive to the effects of the drug may suggest an underlying impairment. Also as previously mentioned a potential flaw in passive avoidance paradigm is that they rely solely on motor function to assess memory which can be problematic as any drug can produce general effects that may result in changes in motor ability.

While the findings from object recognition tasks (Morley et al., 2001; Piper & Meyer, 2004; Piper et al., 2005) concur with those of the current thesis as both have found evidence of MDMA exposure producing significant impairments in performance, it should be noted that these tasks are quite different to the one utilised in the current thesis. Object recognition tasks do not involve learning per se as often these studies use only one trial. Instead they examine the natural disposition of rats to explore a novel object over a familiar one (Ennaceur & Delacour, 1988). Therefore these tasks are quite unique in comparison to other cognitive tasks. They are used to assess memory as when faced with a familiar object and a novel object the subject is inferred to remember the old object if it spends more time exploring the novel object (Ennaceur & Delacour, 1988). However they differ from other memory tasks such as DMTS type tasks that use lots of trails per session or maze tasks that generally utilise multiple training sessions. Hence object recognition tasks may assess different cognitive processes than other cognitive tasks. Despite the evidence that they show disruption with chronic MDMA exposure which concurs with the findings from the current thesis they are difficult to more directly compare with the findings from the partially baited radial maze due to their differing task structure.

There are also a number of studies that have examined the effects of chronic MDMA exposure on the ability to acquire various operant based tasks. For example Li et al. (1988) and Byrne et al. (2000) investigated acquisition of a DRL task. Neither study found significant evidence of binge MDMA exposure disrupting acquisition of these tasks. However it could be argued that these are not complex tasks and are not used to assess cognitive function as they involve simply learning to press a lever at a specific rate and do not require memory processes to the same extent as DMTS or maze type tasks. Hence these tasks may not be complex enough to show evidence of impairments involving higher cognitive processes.

Perhaps the most relevant research for the purpose of this thesis, in terms of similarity of paradigm, are studies that have used other maze tasks. Not all studies that have utilised maze based tasks have found evidence of MDMA exposure producing cognitive impairments. For example Slikker et al. (1989) and Ricuarte et al. (1993) both found no evidence of impairment in acquiring tasks involving various mazes following binge and chronic MDMA exposure despite finding evidence of reductions in 5-HT levels within the brain. However one major difference between Slikker et al.'s (1989) study and that of the current thesis was the route of drug administration. Slikker et al. (1989) administered MDMA via oral gavage whereas the current thesis used intraperitoneal injections. Administering MDMA via injections, either intraperitoneal or subcutaneous are a more common method used in MDMA research despite the fact that most Ecstasy users take the drug orally (Finnegan et al., 1988). This may be important as it is known that the route of administration of a drug can affect absorption with oral drug administration potentially having less of an effect than when the drug is injected (Finnegan et al., 1988). Also of note in Ricaurte et al.'s (1993) study acquisition training within the T-maze did not actually begin until 7 weeks after drug treatment. Therefore these findings are similar to the results from Study 3 that failed to find evidence of a learning impairment in the radial arm maze when there was an eight to ten week gap between drug exposure and training and this may account for the lack of an impairment in performance in Ricaurte et al.'s (1993) study.

Nevertheless, there are a number of studies (Robinson et al., 1993; Broening et al., 2001; Williams et al., 2003; Sprague et al., 2003; Vorhees et al., 2004; Skelton et al., 2006; Able et al., 2006; Skelton et al., 2008; Skelton et al., 2009) that have used Morris water maze and Cincinnati water maze procedures and have found convincing evidence of cognitive impairments produced by chronic and binge MDMA exposure. These results concur with the findings of the current thesis as the studies examined the

acquisition of these tasks and found MDMA treated subjects showed deficits in their ability to learn the tasks compared to saline controls. Like the findings of the current thesis that found MDMA exposure did not affect trial completion time these studies also did not find evidence that the impairments were due to motor impairments as swimming ability remained unaffected. In addition these studies have found that reference memory processes appear to be impaired by MDMA exposure while cued learning and working memory processes remain unaffected which is consistent with the findings of the current thesis.

Longevity of MDMA Induced Cognitive Impairments

There are some studies that suggest that impairments produced by MDMA exposure are transient. For example Robinson et al. (1993) found MDMA treated rats were initially impaired at acquiring a water maze task but eventually were able to learn the task. In addition Vorhees et al. (2004) found the order in which subjects were administered cognitive tasks produced different results. The subjects that were administered MDMA and then trained on a Morris water maze task first (42 days post drug exposure) and then a Barnes maze showed significant impairments in learning the Morris water maze whereas those that were trained on the same Morris water maze task second (77 days post drug exposure) did not produce significant learning impairments. Vorhees et al. (2004) explained this finding suggesting that transfer of learning may have occurred between the cognitive tasks. However another explanation may be that the deficits produced by MDMA were transient and subjects that did not begin training until 77 days after drug treatment may have experienced recovery of function. In contrast other studies have found that MDMA induced cognitive deficits may be more long-term in nature with cognitive impairments being present 50 days

post drug treatment (Williams et al., 2003; Broening et al., 2001). There is also evidence of impairments having been found 160 days post drug treatment and even up to 340 days after drug exposure (Skelton et al., 2006).

The findings from the current thesis as to the longevity of cognitive impairments produced by the regime of MDMA used are mixed. The finding that learning impairments were only found when there was a three day gap between drug exposure and training may indicate that these learning impairments were transient. In other words as subjects were able to acquire the task towards the end of training it might suggest that they no longer were impaired. Therefore it could be argued that the only reason any impairment was found in Studies 4 and 5 was due to the short time delay between drug exposure and training. Whereas in Study 3 when a longer delay (eight to ten weeks) between drug exposure and training occurred there was no evidence of a learning impairment.

In contrast to the findings from Study 3 numerous other maze studies have found evidence of cognitive impairments produced by MDMA exposure with large delays between drug exposure and training. One possible important difference between the studies in the current thesis and previous maze research is the amount of time between drug administration and the commencement of training. The majority of studies that have used the Morris water maze and Cincinnati maze tasks have conducted developmental studies in that they have examined the effect of administering large chronic regimes of MDMA to young rats and then investigated what affect this has on learning when they are older. Hence they leave a large amount of time between drug exposure and training. A possible explanation for the conflicting findings was the age of our subjects who were older than those used in the developmental studies. The rationale of the development studies is to administer the drug at a pivotal developmental stage to examine whether this will produce cognitive

deficits later in life (Broening et al., 2001). However this may not be the finding for studies that utilise adult rats like those conducted in the current thesis as they are not exposed to the drug during this potentially crucial developmental phase where subjects may be more vulnerable to the effects of the drug. However an alternative explanation is the sheer volume of drug that subjects in the developmental studies are exposed to which is much larger than that used in the current thesis.

Other studies within the MDMA literature have also found evidence of cognitive impairments with short time delays between drug exposure and cognitive testing. For example Able et al. (2006) used adult rats and maze training commenced one week after drug exposure and still found evidence of learning reference memory impairments. Similarly Sprague et al. (2003) used adult rats and only had a week long gap between drug exposure and testing in the Morris water maze. Sprague et al. (2003) also found that the rats administered MDMA produced evidence of reference memory impairments which is consistent with the findings of the current thesis. Therefore there is evidence within the literature that suggests cognitive impairments can be produced by MDMA exposure when there is a relatively small time period between drug exposure and cognitive testing which concurs with the findings of Study 4 and 5 of the current thesis. Hence when taking this previous research and the current thesis findings into account there is converging evidence that chronic or binge MDMA exposure produces impairments in acquiring maze tasks. More specifically these impairments seem to involve reference memory processes.

Perhaps the most convincing evidence that the impairments produced by MDMA exposure in the current thesis are not short-term is the findings from the reversal phase in Study 4. In this experiment MDMA treated subjects were significantly slower to acquire the radial maze task compared to saline controls. However they were able to eventually perform at a similar level to controls and

therefore towards the end of this phase of the experiment there was no evidence of a deficit in performance in the MDMA treated animals. After the completion of this phase the rules of the task were reversed and the subjects had to adapt their behaviour and go to previously non-reinforced maze arms and avoid the previously reinforced arms. It was found that MDMA treated animals were significantly worse at this task compared to the controls. Therefore even though the evidence of the initial impairment was no longer visible there was evidence of a continuing underlying impairment in terms of a cognitive flexibility deficit. Therefore the impairments produced by MDMA exposure may be long-term in nature but these deficits become less obvious over time. This may help explain the conflicting findings within the literature where some studies have found evidence of chronic MDMA exposure produced cognitive impairments while others have not.

Type of MDMA Regime Used

Another important difference between the developmental studies and the studies in the current thesis is the amount of MDMA that subjects are exposed to. Typically developmental studies expose subjects to a much more MDMA than that used in the current thesis. In addition the drug is often administered over a much longer period. The most commonly used (Broening et al., 2001; Vorhees et al., 2004; Skelton et al., 2006; Williams et al., 2003) chronic regime in the developmental studies involves 20 mg/kg given twice a day for ten days. Therefore a possible reason why these studies still find evidence of learning impairments after a large time gap between drug administration and training is due to the sheer volume of the drug they have been exposed to which may impair recovery of function.

Indeed the binge regime used in the current thesis (4 x 10 mg/kg for one day) generally involves less MDMA than much of the previous work that has examined the effects chronic MDMA exposure on cognition. For example Robinson et al. (1993) gave double the injections of 10mg/kg of MDMA to that used in the current study and both Able et al. (2006) and Skelton et al. (2008) used four injections of 15 mg/kg in a day. In addition the developmental type studies mentioned above (Broening et al., 2001; Vorhees et al., 2004; Skelton et al., 2006; Williams et al., 2003) also involve larger amounts of MDMA being administered to subjects.

While exposing subjects to a smaller amount of MDMA may result in a less obvious impairment in performance the regime used in the current study has been shown to produce 5-HT damage (Scanzello et al., 1993) and result in significant behavioural effects (Brennan & Schenk, 2006). In addition there has been criticism that the doses of MDMA previously used in chronic studies are unrealistically large compared to human use of the drug (Baumann et al., 2007). Hence the finding that the current study was able to show evidence of cognitive deficits while administering a smaller regime of MDMA adds to the existing MDMA literature.

The Underlying Cognitive Processes in the MDMA Induced Impairments

Within the current thesis, binge MDMA exposure produced deficits that affected the ability of subjects to acquire the task producing significantly slower rates of learning. Although in the second phase of Study 3 a deficit was found when the task had been acquired this was only produced when an additional regime of MDMA was administered. Therefore the current thesis found binge MDMA exposure produced deficits that could be predominantly characterised as learning impairments.

The findings from studies that have used Morris and Cincinnati water mazes along with the results from the current thesis suggests that chronic and binge MDMA exposure can affect cognitive performance. In particular it appears to be learning processes that are more vulnerable to disruption by drug exposure as studies that have examined the acquisition of a task tend to more likely to produce impairments than those that have pre-trained subjects to perform a task and then administered the drug. A possible explanation for this comes from research that has examined the effects of 5-HT lesioning on cognitive performance. For example Cassaday, Norman, Shilliam, Vincent and Marsden (2003) conducted a study that examined the acquisition of two maze tasks and found that subjects in the experimentally 5-HT depleted group were significantly impaired at acquiring the tasks compared to controls. However once the tasks were learnt their performance did not differ from controls. Therefore Cassaday et al. (2003) argued that damage to the 5-HT system produced impairments in acquiring tasks but did not seem to affect performance once the tasks had been acquired. This finding indicates that learning processes may be more easily disrupted than memory processes by 5-HT damage and hence may contribute to the current findings which used a regime of MDMA that has been shown to produce 5-HT damage (Scanzello et al., 1993). However it should be noted that the current thesis did not carry out any physiological measure of 5-HT levels and hence it is unknown whether or to what degree subjects experienced damage to 5-HT levels within the brain.

In particular it appears that reference memory processes seem to be impaired following binge MDMA exposure. There are a number of possible explanations for what is underlying this MDMA induced deficit in reference memory. One possible reason for the impairment in reference memory is that binge MDMA exposure impairs the subjects' ability to encode or input information into long-term memory. Therefore the subjects that have been exposed to MDMA have trouble consolidating the

information about which arms contain reinforcement and hence make a large number of reference memory errors. However subjects do seem to readily learn not to repeat arm visits and hence do not make a large number of working memory errors, thus it appears MDMA exposure does not seem to impair working memory processes.

Another possible explanation for the reference memory deficit is that binge MDMA exposure produced a deficit in acquiring task rules. Therefore subjects have difficulty in ascertaining that the task requires them to learn that there are four arms of the maze that contain reinforcement and four arms that do not. In addition they must learn that this rule does not change between trials or training sessions. Therefore while they do not tend to repeat arm entries due to a natural predisposition to alternative arm entries (Chrobak & Napier, 1992) they do tend to produce a large number of reference memory errors by continuing to visit arms that do not contain reinforcers.

A less cognitive explanation for the behaviour produced by the subjects exposed to MDMA is that the drug simply impairs their ability to utilise extra-maze cues which are generally used to solve radial maze tasks (Liao et al., 2002). However, this explanation would seem unlikely as the visual distortions that are reported with Ecstasy use that could explain these visual impairments are associated with acute use. In other words Ecstasy users only tend to experience visual hallucinations while under the influence of the drug (Peroutka et al., 1988) which has been linked to the increase in 5-HT activity produced by acute MDMA administration (Liechti & Vollenweider, 2000). This makes it unlikely to be a factor in the current thesis as the regime of MDMA used has been shown to produce a decrease or depletion in 5-HT activity (Scanzello et al., 1993).

During training there was no particular evidence that MDMA exposure affected trial completion time. Therefore there did not seem to be any major differences

between MDMA treated rats and controls in terms of how long it took them to complete trials within the radial maze. Hence it seems unlikely that the impairments found after MDMA exposure were the result of drug induced motor impairments. This finding is consistent with the previous research that has found that MDMA has produced reference memory impairments in maze paradigms without affecting swimming ability as assessed by straight channel swimming tasks (Broening et al., 2001; Vorhees et al., 2003; Williams et al., 2003; Able et al., 2006; Skelton et al., 2006; Skelton et al., 2009) which are used to assess motor impairments after drug exposure. Therefore it would appear unlikely that reference memory deficits can be explained in terms of the drug impairing motor ability.

Tolerance and Sensitisation

The findings from the first two studies that examined binge MDMA exposure in the radial maze both found evidence of drug tolerance. This was evident in that rats who were exposed to binge MDMA regimes were less impaired when later exposed to acute doses of MDMA compared to saline controls that has not had previous MDMA exposure. This is consistent with a number of studies such as LeSage et al. (1993), Marston et al. (1999), Frederick et al. (1995), Frederick and Paule (1997) and Frederick et al. (1988) that found the effects of MDMA on cognitive performance lessened with repeated exposure.

However the findings from the final study of this thesis are more complicated in that while there was initial evidence of drug tolerance this effect seemed to dissipate as training continued. Therefore it could be argued that this effect was not driven by drug tolerance but by a floor effect. This occurred as initially acute exposure did not appear to affect the binge MDMA treated rats as performance was so poor that the

addition of further MDMA exposure did not seem to disrupt performance to a notable extent. As training continued as the performance of this group improved the acute doses of MDMA appeared to have more of an effect on performance as accuracy was able to decrease more due to the more accurate level of performance. In fact it appeared that in the final study there may be evidence that acute MDMA exposure had more of an effect on the pre-exposed MDMA group as there was a residual effect of the drug on performance on the days after acute drug administration.

The finding from Study 5 that repeated MDMA exposure while subjects were still learning the task did not produce behavioural tolerance may indicate that learning processes (what subjects use while acquiring the task) are more easily disrupted than memory processes (what subjects use once they have acquired the task) with MDMA administration. In addition the finding that previous binge MDMA exposure produced residual drug effects may also support this suggestion as performance seems to be more disrupted during acquisition with repeated MDMA exposure.

Future Study

As the findings from the second part of this thesis suggest that learning processes (task acquisition) is significantly affected by MDMA exposure, it might be interesting for future research to examine the effects of binge MDMA exposure on acquiring a DMTS task. This is due to the finding that the previous research (LeSage et al., 1993; Taffe et al., 2001; Frederick et al., 1995; Frederick & Paule, 1997) that has examined the effects of pre-training subjects on DMTS and DNMTS type tasks and then exposing them to a chronic and binge MDMA regimes have generally failed to find evidence of cognitive impairments. In addition to replicate and extend the findings from Study 4 from this thesis once the DMTS task has been acquired it could be

interesting to then switch the rules of the task to a DNMTS task. This would allow the longevity of any impairment found to be examined and also assess cognitive flexibility. This study would allow not only acquisition to be studied but also whether any impairment would be found in the ability of subjects to change their behaviour following a change in the requirements of the task which could further support the findings from the current thesis. In addition conducting this research might clarify whether the reason DMTS type tasks fail to produce impairments following MDMA exposure is due to their ability to greater assess working memory rather than reference memory processes or whether the lack of an effect is due to the fact subjects are tested after the tasks have been acquired.

To further extend the results of the current thesis it may be beneficial to replicate the findings using another cognitive task that allows the simultaneous assessment of reference and working memory processes. For example the holeboard task (see Acute MDMA Discussion or van der Staay et al., 1999 for detail on procedure) also assesses spatial memory and allows simultaneous investigation of both working and reference memory processes. A future study could administer the same binge regime of MDMA used in the current study and examine whether acquisition of this task is impaired and whether reference memory processes were more impaired than working memory processes. In addition this study could further examine the effects of MDMA on cognitive flexibility through altering the rules of the task by switching which holes contain reinforcement and examine how MDMA treated subjects alter their behaviour to this change.

Conclusion

In summary the current thesis found binge MDMA exposure resulted in significant impairments in acquiring the partially baited radial arm maze but only if there was a small delay between drug exposure and training. While the learning impairment may have appeared transient and amenable to behavioural recovery it should be noted that when the rules of the task were reversed subjects again showed a significant learning impairment. Hence the MDMA induced impairment may be long-term in nature and primarily involve reference memory processes leaving working memory processes intact. In addition the impairments found seem unlikely to be explained in terms of motor impairments due to a lack of an effect on the time taken to complete trials. Possible explanations for the cognitive impairment found may be the result of a deficit in encoding information into long-term memory or an impairment in acquiring task rules. Unfortunately the current thesis is unable to differentiate between these potential explanations and further research will be needed to determine the nature of the MDMA induced reference memory impairment.

In addition this section of the current thesis replicated the first half of the thesis whereby acute administration of MDMA impaired accuracy, increased trial completion times and resulted in more reference memory errors than working memory errors. Mixed evidence of behavioural tolerance was found whereby Study 3 and 4 produced evidence of behavioural tolerance to acute challenges of MDMA after the task had been acquired. However in Study 5 due to a possible floor effect no convincing evidence of behavioural tolerance was produced. The findings from this half of the thesis suggest that binge MDMA exposure may affect learning processes more so than memory processes. This is because when compared to previous research it would seem that the drug disrupts performance while subjects are acquiring cognitive tasks as opposed to producing impairments once the tasks have been learnt.

In conclusion the second half of the current thesis that examined the effects of a binge regime of MDMA added to the existing literature by utilising a smaller, and possibly more realistic, regime of MDMA than those used in previous research and also utilised a paradigm not used within the MDMA literature that allowed simultaneous assessment of working and reference memory processes. In addition it allowed the assessment of cognitive flexibility by changing the rules of the task which may contribute to the issue of the longevity of MDMA induced cognitive impairments. Finally in Study 5 the effects of repeated acute MDMA administration after an initial binge regime of MDMA were examined. This manipulation has not been conducted by earlier research and produced the interesting finding that it further impaired task acquisition and produced residual drug effects.

GENERAL DISCUSSION

Recap of Findings

The first half of the thesis not only examined the acute effects of MDMA on maze performance but also investigated which neurotransmitter system may be responsible for the memory deficits seen with acute MDMA administration in the partially baited radial arm maze. Study 1 administered the 5-HT agonist Citalopram and the dopamine agonist GBR12909 and an acute dose of MDMA to rats. The dopamine agonist produced a pattern of impairments that were more similar to those produced by MDMA than Citalopram. Therefore, increased dopamine activity when subjects are administered acute doses of MDMA may play more of a role in the reference memory impairments in the radial maze. In Study 2 the D_1 agonist A63930 and the D_2 agonist Quinpirole were administered to examine which dopamine receptor system may be driving the reference memory impairment seen with acute MDMA exposure. At the doses used both agonists produced some level of impairment however when co-administered they produced a synergistic effect more similar to that seen with acute MDMA exposure. Therefore it would appear that not only is it the dopamine release that produces the reference memory effect but that both D_1 and D_2 receptor systems are involved.

The second half of the thesis examined whether binge doses of MDMA would impair the ability of rats to acquire the partially baited radial arm maze task. Learning was significantly impaired when there was a small delay between drug exposure and training. In addition when the rules of the task were reversed subjects again showed a significant learning impairment suggesting there may be long-term underlying

cognitive impairments not readily visible until the requirements of the task are altered. Once again the impairment involved reference memory processes leaving working memory processes relatively intact. In addition in the final study (Study 5) subjects were exposed to binge doses of MDMA and then once a week administered acute doses of MDMA. Task acquisition was significantly impaired with the task taking twice as long to acquire than in Study 4 suggesting repeated MDMA exposure further impairs learning processes. The second half of the thesis also examined whether drug tolerance or sensitisation would occur when subjects were challenged with acute doses of MDMA. Study 3 and 4 found evidence of drug tolerance as when subjects had acquired the task and were challenged with acute doses of MDMA the subjects who had previously been exposed to the binge doses of MDMA were less impaired than saline controls. In Study 5 no convincing evidence of tolerance was found possibly due to a floor effect in performance while subjects were still acquiring the task.

Therefore the current thesis found acute MDMA exposure significantly impaired performance in the radial arm maze by affecting reference memory processes more than working memory processes. In addition binge exposure to MDMA also impairs performance in the radial maze paradigm where learning is significantly slower that is the result of an increase in reference memory errors not working memory errors. Hence the current research found MDMA exposure in general affects reference memory processes more so than working memory processes.

Defining Reference Memory: Problems Comparing Human and Animal Research

One complication when trying to interpret the findings on the effects of MDMA on memory functioning is that the definitions for different memory processes vary between the animal and the human literature. For example working memory in animal

research generally refers to remembering a stimulus during a delay (where this stimulus is not present) after which time a subject must make a response (Dudchenko, 2004). It is a memory process that involves temporary information that is only relevant for a specific trial (Dudchenko, 2004). Hence it involves remembering short-term episodic memory that involves stimuli or events they have just seen or places they have just been (Hampton & Schwartz, 2004). For example whether the subject was just presented with a red or green light or which arm of a maze it just entered.

However within human research the definition of working memory is more complex. It arose from an earlier construct called short-term memory (Baddeley, 1998) that involved a unitary system that temporarily stored information (Baddely, 2000). The term working memory now generally refers to Baddely's three component model that involves the temporary storage and manipulation of information needed to perform complex tasks such as learning, memory and comprehension (Baddeley, 2000). More generally within the human literature it refers to the ability to temporarily store and manipulate information (Howard et al., 2003).

This type of memory functioning is typically more complex than that referred to as working memory within the animal literature and hence could be explaining an entirely different set of memory processes. Indeed even Baddeley (2000) has argued the term working memory used within animal research involves the storage of information over several trials and to some extent may rely on long-term memory processes. Hence Baddeley (1998) argued that animal working memory processes involve different memory processes than those used in human literature (Baddeley, 1998).

To make matters even more complicated the definition of exactly what is involved in reference memory remains unclear even within the animal literature. Most

papers that examine reference memory define it solely in terms of the apparatus used. For example in the partially baited radial maze reference memory is defined as visiting an arm that does not contain reinforcement (Olton & Papas, 1979). Whereas in the Morris water maze it is defined as the ability of a subject to learn where a platform is within a water maze that does not change position from trial to trial (Frick et al., 1995). These papers often do not delve into any further explanation or discussion on what the reference memory processes involved in these tasks entail. Studies that have tried to define reference memory have described reference memory as a long-term, (Dudchenko, 2004) trial independent form of memory as the to be remembered information remains the same across trials (Olton & Papas, 1979). It has also been described as the memory process involved in acquiring task rules, for example running to the end of a maze or swimming to a fixed platform (Frick et al., 1995).

Even within the animal literature there appears to be some debate as to how to assess reference memory. For example there appears to be confusion as to whether tasks that involve visual discrimination that involve a task rule actually assess reference memory or whether it involves more of a memory (having to remember information) component. For example Bushnell and Levin (1993) used modified DMTS task trials involving visual discrimination that they argued assessed reference memory. In these trials subjects had to press the lever in an operant chamber which illumined a light source above it. Hence subjects did not have to remember which lever to press from trial to trial they only had to remember the general rule that they needed to press the lever with the light above it. However it could be argued that this task does not really encompass the complexity of reference memory processes as it did not involve a memory component. Hence this task was more similar to cued learning type tasks. So even though the definition of reference memory is often described as a long-term general rule that does not change from trial to trial it does seem more complicated

in that the subject has to remember something from trial to trial that is not externally cued. Therefore reference memory functioning appears to involve more complex cognitive processes than simple visual discrimination or cued learning as it appears to involve a larger memory component in that subjects are required to remember spatial information across trials.

Another problem when trying to make comparisons between human and animal research is that there appears to be no real definition of reference memory in human literature. This makes trying to determine what the equivalent memory process in human memory functioning difficult. The closest definition for reference memory in the human cognitive literature appears to be that of procedural memory which involves the gradual acquisition of rules or procedures (Thomas-Ollivier et al., 1999). Procedural memory also is defined as the processing system responsible for the encoding, storage and retrieval of procedures that can be motor or cognitive and hence have been examined using tasks assessing the learning of both motor and cognitive tasks (Beaunieux et al., 2006). Unfortunately there is no apparent research that has examined the effects of MDMA on this type of human memory functioning. In addition procedural memory often tends to refer to basic motor or perceptual learning involving tasks such as mirror reading, pursuit rotor tasks and jigsaw puzzles (Schmand, Brand & Kuipers, 1992). This is problematic as this type of learning does not encompass the type of reference memory referred to in animal studies. More recently there has been more focus on cognitive procedural memory which involves more complex cognitive rule learning which uses problem solving tasks (Schmand et al., 1992).

Hence cognitive procedural memory is similar to human working memory and executive functioning as it assesses the ability of subjects to learn cognitive task rules. For example disc transfer tasks like the Tower of Hanoi and Tower of London are used

to assess executive functioning and cognitive procedural memory (Schmand et al., 1992; Beaunieux et al., 2006). These tasks involve three poles that have a varying number of disks placed on them (Schmand et al., 1992). In general the task involves having to move the disks from the first pole to the last pole as quickly as possible and in as few moves as possible by only moving one disk at a time and not placing a bigger disk on top of a smaller one (Schmand et al., 1992). These tasks have been argued to assess executive functioning over the initial trials and assess cognitive procedural learning when they are administered over several sessions (Beaunieux et al., 2006). Fox et al. (2001) found Ecstasy users were impaired on the Tower of London task. However only twelve trials were given during this study and therefore executive functioning alone may have been measured as opposed to cognitive procedural memory. It would be interesting for future studies to examine whether Ecstasy users are impaired on these types of tasks when they are administered over repeated sessions.

The current thesis found strong evidence that MDMA exposure affects reference memory processes as measured by the partially baited radial maze paradigm. But it is difficult to claim with certainty what processes Ecstasy users may show corresponding impairments in due to the discrepancies between the definitions and types of memory processes used in animal and human cognitive literature.

The Underlying Cognitive Impairments of the Reference Memory Effect

One aim of this discussion is to try to find an underlying cognitive impairment that will tie the findings of the two parts of the thesis together. This is not an easy ask considering the acute and binge regimes used produce very different physiological responses. The findings from the first half of this thesis that involved acute MDMA

administration suggest that the reference memory impairments produced by MDMA exposure are the result of the release of dopamine. However, the findings from the second half of the thesis suggest that the reference memory impairments found are the result of a decrease in 5-HT activity as the regime we used has been shown to reduce 5-HT levels (Scanzello et al., 1993).

One of the most clear and replicated findings from the current thesis is that MDMA exposure (whether acute of binge) appears to affect reference memory more than working memory processes as measured by the partially baited radial maze. One difficulty in interpreting these findings is in trying to ascertain what exactly reference memory is and what the underlying cognitive processes are that produce the pattern of errors that has been found in the partially baited radial arm maze. Reference memory errors involve subjects going to arms of the maze that do not contain reinforcers rather than repeating arm visits within a trial (working memory error). Thus the question remains as to why MDMA produces this particular pattern of behaviour within the maze.

One possible explanation that has been given within this thesis is that MDMA exposure may produce long-term memory impairments. With acute MDMA studies (where they task has been acquired) the drug may impair the ability to retrieve information from long-term memory about which arms contain reinforcement and hence rats visit arms that do not contain reinforcers. During the binge studies while rats are still acquiring the task the drug may impair the ability of subjects to encode the information about which arms contain reinforcement into long-term memory. They do not tend to make working memory errors as this form of memory is left intact and they do not repeat arm entries within a trial. Therefore the rats can remember where they have been in a trial (avoiding working memory errors) but are unable to retain their

memory of where they have obtained reinforcers across trials (producing reference memory errors).

An alternative explanation is that MDMA exposure produces impairments in acquiring or using task rules. In acute studies when MDMA is administered subjects may become confused as to what they are meant to be doing. They are able to remember where they have been and hence do not repeat arm entries into already visited arms but they have difficulty ascertaining that there are arms of the maze that contain reinforcers and those that do not. In addition during the binge studies where subjects have already acquired the task the subjects may also become confused as to the rules of the task after MDMA exposure. Hence while learning the task subjects have difficulty ascertaining that the maze arms that contain reinforcers do not change from trial to trial. Subjects may not make working memory errors as when drugs are administered and performance is disrupted they fall back on their naturally occurring behaviour which is going to novel arms (not repeated arms). Rats have an innate disposition for preferring novelty or to alternate (not go back and revisit arms). Unfortunately neither of these explanations are easily disentangled from one another. Also when learning long-term unchanging rules it would be difficult not to involve long-term memory so these two explanations may be unable to be differentiated.

In addition, in the Acute Interim Discussion it was argued that the reference memory impairment seen with acute MDMA exposure was unlikely to be the result of perseveration. This was because if rats were going to perseverate they would be more likely to produce more working memory errors than reference memory errors as they would repeatedly visit arms. However the MDMA induced reference memory impairment seen with acute drug exposure may be able to be explained via perseveration if instead of thinking in terms of individual or single arm entries being the response rather subjects solve the task by producing a sequence of arm visits.

Instead of each arm visit being a response, all four arm visits (in a particular sequence) are the response. Indeed once subjects had acquired the task they tended to stick to a fixed arm entry pattern whereby they would enter the reinforced arms of the maze in a specific order. Perseveration may occur not by repeatedly visiting a particular arm but by repeating a particular arm entry pattern consisting of a set of arm entries. Hence subjects learn the correct arms of the maze as a sequence and they repeat it from trial to trial. When they are given MDMA if they make a mistake early on in the sequence they do not self-correct and continue with their fixed sequence despite it not producing reinforcement. They continue to produce a fixed behaviour (set of arm entry patterns) despite it not being effective and show a preservative pattern of behaviour. This pattern of responding would result in reference memory errors but not working memory errors as subjects would not repeat arm entries but instead mistakenly go to unbaited arms of the maze.

The impairments found in the binge studies could also be explained by subjects producing a perseverative pattern of responding. During habituation trials at the beginning of all studies all arms of the maze are baited and the rat is allowed to explore the maze. This was done to ensure that the rats would visit all arms of the maze and not adapt any biases where they might avoid certain arms of the maze. However rats may tend to adopt a strategy where they visit all arms of the maze by simply learning to go to consecutive arms. This pattern of responding would produce very few or no working memory errors as they would simply keep going to the next arm of the maze until they had consumed all reinforcers at which point they were removed from the maze. When actual maze training began only four arms of the maze are baited, however some rats may continue to adopt this strategy. Specifically when they are only allowed four arm entries they may enter four consecutive arms of the maze before being removed and then when placed back in the maze for the next trial they visit the other four arms of

the maze. Hence they adopt a particular strategy to solve the task before they learn that some arms contain reinforcement while others do not. As MDMA exposure may result in perseveration the rats that were exposed to MDMA may continue to adopt this strategy where they try to visit all arms of the maze instead of trying to ascertain which arms of the maze contain reinforcers. Thus they produce more reference memory errors and take significantly longer to learn the partially baited maze task than controls.

Unfortunately the data collected from these experiments does not easily lend itself to these arm entry pattern analyses. This could be an interesting area of research to explore in future studies where an experimental design that examined arm entry patterns and sequence learning could be established. Indeed it has been shown that tasks involving lever pressing sequences have been impaired with acute MDMA exposure (Frederick & Paule, 1997) with subjects showing a perseverative pattern of responding. However Winsauer et al. (2002) failed to find a difference in performance before and after chronic MDMA treatment in a repeated acquisition lever pressing task suggesting MDMA exposure did not affect this form of learning. It should be noted that Winsauer et al. (2002) gave subjects experience with the task before drug exposure and hence performance may have been harder to disrupt. This pattern of responding could also be used to explain some of the patterns of impairment found with MDMA administration in the existing literature. For example the finding that rats are affected by the previous trial type in DMTS tasks (Harper et al., 2005; Harper et al., 2006) may suggest a perseverative impairment rather than proactive interference. Harper et al. (2005; 2006) found if a rat that had been administered acute MDMA pressed on the left lever in a trial it was then more likely to continue responding on the left lever thus suggesting a perseverative pattern of behaviour. Indeed perseveration is a pattern of responding that has been found in Ecstasy users (von Geusau et al., 2004; Smith et al., 2006; Montgomery et al., 2005; Dafters, 2008; Verrico et al., 2008).

Therefore a possible explanation for the impairments seen with MDMA exposure may be the result of MDMA administration producing perseveration.

Tolerance/Sensitisation

The findings from Study 3 and 4 both found evidence that subjects that had been administered binge regimes of MDMA were less impaired than saline controls when later challenged with acute doses of MDMA. Hence these studies provided evidence that repeated MDMA exposure resulted in behavioural tolerance where subsequent exposure to MDMA has less of an effect on performance. This is a consistent finding within the animal literature that has found that the effects of MDMA reduce with repeated exposure (LeSage et al., 1993; Marston et al., 1999; Frederick et al., 1995; Frederick & Paule, 1997; Frederick et al., 1988). In addition there is evidence within the human literature that Ecstasy users report tolerance developing with repeated drug exposure (Parrot, 2001; Cottler et al., 2001; Parrot, 2005).

In Study 3 and 4 subjects had acquired the task before they were challenged with acute drug exposure. However in Study 5 subjects were repeatedly challenged with acute drug exposure while they were still acquiring the radial maze task. In Study 5 convincing evidence of drug tolerance was not found as there were no obvious differences between saline and binge MDMA treated animal's performance when they were challenged with acute doses of MDMA. One possible explanation for this finding is that the performance of the binge treated MDMA group was so poor that is was unable to show evidence of impairment with acute MDMA administration. Hence this finding may be due to a floor effect.

Another possible explanation is that while subjects are still acquiring the task they are more susceptible to the disruptive effects of MDMA exposure suggesting

learning processes are more affected by MDMA exposure than memory processes which would be more required once task acquisition occurred. Of interest acute MDMA exposure seemed to have more of an effect on the binge treated group as there was evidence of residual drug effects the day after acute drug administration which was not present in the saline treated group.

Implications for Human Ecstasy Users

As stated earlier in this chapter due to the lack of research examining reference memory processes per se within the human literature, it is difficult to extend the current findings of this thesis to human Ecstasy users. However, when taking the findings from the first half of the current thesis that examined the acute effects of MDMA on cognitive performance it could be speculated that while under the influence of Ecstasy people may experience difficulties in performing previously well learnt cognitive tasks. It has been reported that when acute doses of Ecstasy were administered to healthy volunteers they reported difficulty in concentrating, decision making and general disturbances in thinking (Vollenweider et al., 2002) indicating that the drug could affect the ability of users to perform cognitive tasks.

In addition within the second half of the thesis that examined binge regimes of MDMA on task acquisition it was found that subjects who were exposed to MDMA showed slower rates of learning than controls. Hence Ecstasy users may show slower rates of learning on cognitive tasks. Again there is some evidence of this within the literature showing Ecstasy users are impaired at acquiring cognitive tasks compared to controls. For example Fox et al. (2001) found Ecstasy users required significantly more trials to perform at a similar level to controls on a task that assessed verbal learning. Verbal learning has also been found to be impaired in Ecstasy users in other

studies (Reneman et al., 2001; Gouzoulis-Mayfrank et al., 2000; McCardle et al., 2004). McCardle et al. (2004) suggested the impairment in verbal learning was the result of Ecstasy users having deficits in encoding information into long-term memory which would be consistent with the findings of the current thesis. In addition Ecstasy users have also been found to show associative learning impairments requiring significantly more trials to acquire an associating learning task (Montgomery et al., 2005) which is also consistent with the findings of the current thesis.

Similarly due to the findings in Study 4 where subjects who were exposed to MDMA took longer to change their behaviour when the rules of the task were altered may suggest that Ecstasy users may show impairments in cognitive flexibility. Consistent with this finding Ecstasy users have produced impairments in tasks that assess the ability to change performance in the face of changing consequences (von Geusau et al., 2004; Smith et al., 2006; Montgomery et al., 2005; Dafters, 2008; Lamers et al., 2006).

Future Research

Trying to differentiate between the explanations for what is the underlying cause of the reference memory impairments would be beneficial. In particular future research examining the perseveration explanation of the reference memory impairments could be useful. Hence a future study could examine the sequence of arm visits with acute drug administration. For acquisition studies using binge regimes of MDMA it could be interesting to examine whether starting maze training with only four arms of the maze being baited (excluding the habituation phase where all arms are baited) would affect the rate of learning in MDMA treated animals. In addition it would be helpful to try and replicate the findings of the current thesis by using another

paradigm that allows working and reference memory processes to be examined simultaneously. Therefore the holeboard task could be a useful paradigm to further study the effects of MDMA on working and reference memory processes. Finally in order to ascertain whether previous studies have failed to find evidence of binge or chronic regimes affected DMTS performance future study could examine the effects of binge regimes of MDMA on acquiring the DMTS task. This is because previous studies have trained subjects on the tasks before drug administration and have not examined the effect of the drug on DMTS acquisition.

Conclusion

In summary the current thesis found both acute and binge MDMA exposure produced impairments in the partially baited radial arm maze. Reference memory processes were more adversely affected than working memory processes. Also there was some evidence of behavioural tolerance occurring when binge regimes of MDMA were administered and subjects were challenged with acute doses of MDMA after the task had been acquired. However no clear evidence of tolerance was found with prolonged repeated MDMA exposure while task acquisition was occurring suggesting MDMA exposure may disrupt learning processes.

Of note, the findings from the current thesis are interesting in that working memory processes are usually described as more prone to interference whereas reference memory processes are considered to be harder to disrupt (Olton & Papas, 1979). By using the partially baited radial arm maze that allows the simultaneous differentiation between working and reference memory the current thesis found exposure to MDMA predominantly disrupted reference memory processes leaving

working memory relatively intact. Hence this is a relatively novel finding that shows the drug disrupted a memory process that is generally thought to be more robust than working memory and therefore adds an interesting counterintuitive finding to the existing MDMA literature.

In conclusion the current thesis examined the effects of acute and binge regimes of MDMA on a paradigm not previously used within the existing MDMA literature that allowed simultaneous assessment of working and reference memory processes. In addition it examined cognitive flexibility by altering the rules of the cognitive task which is not commonly performed in the existing literature. This thesis also examined the effects of repeated MDMA exposure which is also not commonly performed within the MDMA literature and is important as it more closely mirrors human Ecstasy use. While the current thesis consistently produced the finding that MDMA exposure resulted in more reference memory errors than working memory errors it still remains unclear as to why these pattern of responding emerged. Further research is needed to try and differentiate what underlying cognitive impairments produced the MDMA induced reference memory impairments in the radial arm maze.

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