LONG TERM EFFECTS OF MDMA ADMINISTRATION IN
RATS DURING EARLY AND LATE ADOLESCENCE

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of Master of Science in Psychology

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<tbody>
<tr>
<td>Degrees</td>
<td>Degrees</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>5-hydroxyindoleacetic acid (serotonin)</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine (serotonin receptor)</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>C</td>
<td>Celcius</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeters</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>DA</td>
<td>Dopamine</td>
</tr>
<tr>
<td>DOPAC</td>
<td>3,4-dihydroxyphenylacetic acid</td>
</tr>
<tr>
<td>Fig.</td>
<td>Figure</td>
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<tr>
<td>i.p injection</td>
<td>intraperitoneal injection</td>
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<tr>
<td>MDMA</td>
<td>3,4-methylendioxymethamphetamine (Ecstasy)</td>
</tr>
<tr>
<td>mg</td>
<td>Milligrams</td>
</tr>
<tr>
<td>mg/kg</td>
<td>Milligrams per kilogram</td>
</tr>
<tr>
<td>min</td>
<td>Minutes</td>
</tr>
<tr>
<td>ml/kg</td>
<td>Milliliters per kilogram</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate, (glutamate receptor)</td>
</tr>
<tr>
<td>P</td>
<td>Post-natal day</td>
</tr>
<tr>
<td>PND</td>
<td>Post-natal days</td>
</tr>
<tr>
<td>s</td>
<td>Seconds</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>Standard error of the mean</td>
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Abstract

Drug use and abuse for recreational purposes is a common phenomenon, with club drugs such as MDMA (3,4-methylenedioxymethamphetamine) being popular for its energetic and euphoric effects – recreating an artificial feeling of “Ecstasy”. Although use of the drug itself has remained relatively constant over the years, the population among which it is popular has been shifting toward younger users, with MDMA use among adolescents becoming more prominent. However research on the effects that MDMA has on the developing adolescent brain has been limited. The current study focuses on the long term effects in rats following chronic MDMA exposure during either early or late adolescence. In adulthood, the rats’ memory, activity and emotional reactivity were assessed through frequency of ambulation, grooming, rearing, defecation, and corner or center occupancy of an open-field, novel object-recognition in the open-field, emergence from a dark chamber into a bright area, and recognition of the changed arm of the Y-maze. The results showed that there were significant long-term effects resulting in increased anxiety for rats treated with MDMA during late adolescence only. This increase of emotional reactivity was indicated through decreased ambulation on the open-field measures, decreased movement between the dark and light chambers, and decreased entries of both arms of the Y-maze. Sex of the animal was also found to differentiate MDMA effects, with females showing a greater increase in anxiety. Measures regarding spatial and working memory were not significant. Overall, the results suggest that animals are more susceptible to long-term effects following MDMA administration in late, but not early adolescence. Furthermore, memory appears to remain unaffected regardless of the age of administration, and only anxiety levels were affected by the drug.
1.0 Introduction

1.1 GENERAL OVERVIEW

MDMA, or 3,4-methyldioxymethamphetamine is a recreational drug used by partygoers world-wide. It is the main ingredient of the street drug known as “ecstasy”, named for its energetic and euphoric effects caused through the release of brain serotonin and dopamine. Ecstasy is a Class A drug in many countries due to its acute and long-term effects on serotonin, or 5-hydroxytryptamine (5-HT, (Piper, 2007). In New Zealand, MDMA is a Class B controlled drug (Noller, 2009).

In rats, MDMA has been shown to produce a similar effect as in humans: an acute and rapid release of serotonin (5-HT) accompanied by an increased release of dopamine from cerebral tissue (Green, Mechan, Elliott, O'Shea, & Colado, 2003). Research supports that single and multiple administration of MDMA has been shown to result in long-term depletion of 5-hydroxyindoleacetic acid (5-HIAA) and 5-HT (Easton & Marsden, 2006; Green et al., 2003). Neurotoxic effects of MDMA have been found to appear anywhere between 24 hours to a week after MDMA administration (Schmidt, 1987).

There are a number of factors that have been shown to modulate the effects of MDMA, such as age, sex, and dose. The dose administered and the frequency and duration of the treatment often elicit differing results. The age at the time of drug administration appears to have an impact on the outcome. Sex often seems to play a determining factor also, as shown by the few studies that have examined its role on the neurotoxic outcomes following MDMA administration (Easton & Marsden, 2006).
Young adults make up the majority of Ecstasy users, with teenage drug use on the rise. Research on the effects of MDMA is extensive (Green et al., 2003; Parrott, 2001), but the focus lies in adult users of the drug, and does not reflect the growing trend of adolescent drug use and abuse. Given the ongoing development of the brain well into young adulthood it would suggest the effects of MDMA may well be different during adulthood compared to during adolescence.

1.2 MDMA

3,4-Methylenedioxymethamphetamine (MDMA) is an amphetamine derivative developed in 1912 to be used first allegedly as an appetite suppressant, then as an experimental agent for psychological warfare by the US army in the 1950s, a therapeutic tool in psychotherapy in the 1970s, and later as a popular recreational drug (Greydanus & Patel, 2005; Lopez, 2003; Montoya, Sorrentino, Lukas, & Price, 2002; Piper, 2007). Following evidence that it induced serotonergic nerve terminal degeneration, combined with its high abuse potential and lack of clinical application, in America MDMA was consequently classified as a Class A drug in the 1980s (Green et al., 2003; Skelton, Williams, & Vorhees, 2008). In New Zealand, MDMA is classified as a controlled Class B drug (Noller, 2009). Since the 1980s, MDMA has increased in use as a recreational drug at dance parties and in clubs. Commonly known as “Ecstasy”, “Extacy”, “XTC”, “Ecky” or “E”, MDMA is sold in pill form, and although the purity and dose of MDMA content in a tablet of Ecstasy vary, tablets have been found to contain on average between 80 and 150 mg of MDMA (Green et al., 2003; Lopez, 2003). Tablets are usually taken orally, although when in powder form it can also be snorted or injected for faster absorption into the system.
The onset of effects include a relaxed and euphoric state, emotional openness, empathy, reduction of negative thoughts, teeth grinding, jaw clenching, and a decrease in appetite and inhibitions. These can take between 20 to 60 minutes to occur, with the peak occurring 60 to 90 minutes after ingestion, and the primary effects lasting between three to five hours (Green et al., 2003). More serious and potentially fatal acute effects, which are often due to the environment – most commonly constant dancing with little access to water – have been found to result in hyperthermia, dehydration, hyponatremia, aggravation of underlying health conditions, and serotonin syndrome (Noller, 2009).

1.2.1 Biochemical Effects

MDMA is an indirect monaminergic agonist which affects peripheral and central nervous system (CNS) functions (Lyles & Cadet, 2003). MDMA causes a release of serotonin and dopamine (DA) in the brain, and acts indirectly, by stimulating the release and inhibiting the reuptake of serotonin and, to a lesser extent, other neurotransmitters (Montoya et al., 2002; Parrott, 2001). MDMA binds to all three presynaptic monoamine transporters – noradrenaline, dopamine, and serotonin, but has the highest affinity for the 5-HT transporter (Green et al., 2003). Transporter binding levels have been shown to be affected by MDMA use, with decline in the serotonin transporter binding observed (Piper & Meyer, 2004). MDMA administration in rats has been found to induce an acute and rapid release of dopamine and 5-HT in the striatum, nucleus accumbens caudate nucelus and hippocampus (Green et al., 2003; Lyles & Cadet, 2003). There is a significant amount of evidence which indicates that use of MDMA also results in a decrease in dopamine, 5-HT and 5-HIAA in the amygdala, hippocampus and striatum (Faria et al., 2006; Green et al., 2003; Gurtman, Morley, Li, Hunt, & McGregor, 2002; Lyles & Cadet, 2003; Morley-Fletcher, Bianchi, Gerra, & Laviola, 2002; O’Shea, Granados, Esteban, Colado,
& Green, 1998; Skelton et al., 2008). MDMA has also been found to alter norepinephrine release, although it has not been shown to reduce norepinephrene levels in the rat brain (Skelton et al., 2008).

Research indicates that following the initial decrease of 5-HT, concentrations have been found to return toward pre-treatment levels within 24 hours. Following this initial recovery, cerebral 5-HT concentrations have then been shown to decline to as low as 74% of control values one week later, due to specific neurotoxic damage to 5-HT nerve endings in the forebrain (Green et al., 2003; Schmidt, 1987). This neurodegeneration has been demonstrated to last for several months in rats (Green et al., 2003). This is significant, as depletion of 5-HT has been implicated in memory deficits (Montoya et al., 2002). Neuroendocrine systems are also affected, showing an increase in corticosterone 30 minutes after treatment, and back to baseline by six hours after. Similar results have been found for prolactin levels, peaking at one hour after MDMA administration and returning to baseline levels by four hours (Nash, Meltzer, & Gudelsky, 1988; Skelton et al., 2008). Multiple doses of MDMA have been shown to result in a significant reduction in [3H]dopamine uptake (35-55%) one hour post administration, but this effect was reversed with 24 hours post administration (Green et al., 2003; Hansen et al., 2002). Research has shown that whilst all brain regions have been shown to make a complete recovery of both 5-HT and 5-HIAAA content within a year following treatment with MDMA, the rate of recovery was region-dependent (Green et al., 2003).

Reduction in 5-HT levels following moderate to high dose MDMA exposure is the most consistently identified long-term effect (Piper, 2007). Furthermore, animal studies have shown that permanent destruction of serotonergic neurons is possible following depletion of 5-HT early in life (Montoya et al., 2002).
Neurotoxic effects of MDMA appear between 24 hours and one week following MDMA administration (Lyles & Cadet, 2003; Schmidt, 1987). However, it is not MDMA itself which produces neurotoxicity in the brain, but is the result of peripherally formed metabolites (Green, Gabrielsson, Marsden, & Fone, 2009). Contrary to popular opinion, chronic treatment with MDMA is not necessary to develop toxicity, and studies in rats have shown that even a single exposure to MDMA can produce some neuronal damage (Montoya et al., 2002). Toxicity has also been found to result from an excess of serotonin within the CNS, a condition known as the serotonin syndrome and which is potentially fatal (Noller, 2009).

Forebrain structures such as the hippocampus and the frontal cortex are essential for cognitive function, and are highly sensitive to MDMA (Green et al., 2003). The serotonin system in these regions undergoes dynamic and protracted development (Piper, 2007). Treatment with fluoxetine was shown to prevent the dose-related increase in locomotor activity normally produced by MDMA (Callaway, Wing, & Geyer, 1990), which would indicate that release of 5-HT has a strong influence on the behavioural effects of MDMA.

1.2.2 Behavioural Effects

Behavioural effects in rats following MDMA intake include hyperthermia, hyperactivity, and the serotonin behavioural syndrome, the characteristics of which include (among other symptoms) enhanced locomotor activity, head-weaving and piloerection (Lyles & Cadet, 2003). Studies of human subjects also include hyperthermia, in addition to dehydration and hyponatremia, or “water intoxication” – often a result of over-hydration in an attempt by the drug user to prevent dehydration. Furthermore, repeated use of MDMA by human subjects has shown disturbances in sleep and mood, increased anxiety, elevated impulsiveness, memory deficits, and attention problems, all of which may persist for up to two years after cessation of
MDMA use (Montoya et al., 2002). Chronic use of MDMA, defined here as both repeated and heavy doses, was shown to result in an array of cognitive impairments, ranging from memory deficits (including short-term, delayed, visual, verbal, working, and episodic memory, as well as memory for new information) to impairments in central executive functioning and reasoning and semantic recognition (Montoya et al., 2002).

Animal studies have produced conflicting results regarding effects on anxiety following MDMA administration. For up to three months following drug treatment, greater anxiety-like behaviours than controls have been reported in emergence, elevated plus maze, and social interaction tests, along with open-field behaviour and social interaction (Fone et al., 2002; Morley, Gallate, Hunt, Mallet, & McGregor, 2001). The increase in anxiety behaviours was not always accompanied by any measurable neurotoxic loss of 5-HT (Fone et al., 2002). In contrast, other research found conflicting results with an apparently anxiolytic response in rats tested on the elevated plus maze 73 to 80 days after a neurotoxic dose of MDMA (Mechan et al., 2002).

Research shows that MDMA exposure during brain development results in a disruption of sequential and spatial memory-based learning, and that such deficits were developmentally specific (Broening, Morford, Inman-Wood, Fukumura, & Vorhees, 2001; Marston, Reid, Lawrence, Olverman, & Butcher, 1999). Furthermore, these effects were not related to any long-term changes in 5-HT, dopamine, or noradrenalin (Broening et al., 2001; Green et al., 2003).
1.3 MODERATING FACTORS

Species differences in response to drugs can account for different effects observed following drug administration, whether human or rodent data. As it has also been observed that different rat strains appear to have different sensitivities to both the acute as well as the long-term effects of MDMA, the strain used must be taken into consideration (Easton & Marsden, 2006; Green et al., 2003). Furthermore, as mice were used in several studies rather than rats (Maldonado & Navarro, 2000; Morley-Fletcher et al., 2002; Reveron, Monks, & Duvauchelle, 2005), possible species differences in MDMA effects and MDMA-induced damage must also be taken into consideration. Research has shown that the different sensitivities to the neurotoxic effects of MDMA may be due to differences in metabolism of MDMA among different strains of rats (de la Torre & Farré, 2004). This implies that the interpretation of the effects of any specific dose may be dependent on other factors such as species and strains, as it is impossible for all of the administered drug (at any stated dose) to be responsible for the observed pharmacological effect (Green et al., 2009). Nevertheless, it is possible to draw parallels between animal models of MDMA and the potential implications for humans (Easton & Marsden, 2006). However, a number of moderating factors must be taken into consideration when designing an experiment which may be relevant to humans: the dose used, the age of the animal at administration and testing, and the sex.

1.3.1 Dose

Doses for administration are often hard to determine due to the fact that the purity and amount of MDMA varies from pill to pill, although on average, tablets have been estimated to contain between 80-150 mg (Green et al., 2003). However, more recent data indicates that the content of MDMA in Ecstasy has dropped as low as 40 mg (Noller, 2009).
Human MDMA intake tends to be varied in amount and frequency, with the majority of users taking the drug orally during the weekends. As animal studies most frequently administer drugs via intraperitoneal (i.p.) injection this often creates the misconception that animal studies are relevant only to chronic users of MDMA. This makes animal dosing regimens difficult to relate to human drug use, in terms of both dose and frequency of administration (Easton & Marsden, 2006). The outcome of an animal study may very well differ depending on both the dose and dosing regimen, as well as the metabolic rate for that particular species and drug. Studies have shown that a dose of approximately 7 mg/kg of MDMA is required for rats to achieve the equivalent peak plasma MDMA concentration of a 2 mg/kg dose for humans (Green et al., 2009).

Varying strengths of a single dose of MDMA were compared to each other in a number of rodent studies (Maldonado & Navarro, 2000; O’Shea et al., 1998; Palenicek, Votava, Bubenikova, & Horacek, 2005). Doses of 1, 2.5, 4, 5, 8, 10 or 15 mg/kg were found to produce a dose-dependent increase in activity except for 1 mg/kg. Furthermore, although the 4 mg/kg dose showed no effect, a single 10 mg/kg dose caused considerable neurotoxic damage to 5-HT nerve terminals, while a single 15 mg/kg dose produced a greater than 50% loss of 5-HT and 5-HIAA content (O’Shea et al., 1998).

Other investigators investigated repeated administration of a dose of 10 mg/kg at two hour intervals (Faria et al., 2006; Koenig et al., 2005). When it was administered to male Wistar rats every two hours for a total of six hours, long-term depletion of 5-HT was seen ten days following exposure (Faria et al., 2006). However the same dose administered three times at two hourly intervals to male and female Long-Evans rats resulted in the deaths of all of the male animals and three out of ten females following the third injection (Koenig et al., 2005). A dose
of 20 mg/kg administered four times at two hourly intervals to mice showed a decrease of 5-HT, dopamine transporter, and DOPAC, seven days following exposure (Reveron et al., 2005).

A lower dose administered at more frequent intervals was investigated by several researchers (Gurtman et al., 2002; Morley et al., 2001; O’Shea et al., 1998). Five mg/kg of MDMA administered every hour for four hours for two consecutive days produced increased anxiety and memory impairment 9-14 weeks following treatment, as well as a significant decrease in 5-HT and 5-HIAA levels. Furthermore, when 4 mg/kg MDMA was administered twice daily for four days, substantial damage of up to half the control indole concentrations of 5-HT, 5-HIAA, and [³H]paroxetine in the cortex, hippocampus, and striatum was observed, but not when it was administered once daily (O’Shea et al., 1998).

A significant amount of research has been focused on developing an intermittent dosing regimen mimicking human recreational drug use (Meyer, Piper, & Vancollie, 2008; Morley-Fletcher et al., 2002; Piper, Fraiman, & Meyer, 2005; Piper & Meyer, 2004). Intermittent exposure of either 0, 5, and 10 mg/kg of MDMA for three days, with a two day interval between each treatment, was found to induce dose-dependent analgesia in all age groups (Morley-Fletcher et al., 2002). When MDMA was administered at 5 mg/kg hourly for four hours every five days from P35-60, attention was impaired and serotonin transporter binding was reduced (Piper et al., 2005). However, when MDMA was administered at 10 mg/kg twice daily with an interdose interval of four hours every five days from P35-60, cognitive and affective functioning was found to be affected, although only modest decreases in serotonin binding were observed (Piper & Meyer, 2004). Even though both groups had received a total of 20 mg/kg per day, the dosing regimen evidently affected memory and anxiety only at higher initial doses. Following on from this finding, Meyer et al (2008) developed a model of intermittent MDMA exposure, using 10
mg/kg of MDMA, calculated to reflect a human dose. This dose was given twice a day, with four hours in between exposure, every five days, for a total of six treatment days (Meyer et al., 2008).

Other research considered longer periods of exposure (Broening et al., 2001; Mayerhofer, Kovar, & Schmidt, 2001; Wiley, Evans, Grainger, & Nicholson, 2008). A daily dose of 20 mg/kg for a period of ten consecutive days was found to have reduced serotonin and noradrenalin levels two and four weeks following exposure. However, dopamine levels showed an increase four but not two weeks after exposure (Mayerhofer et al., 2001). A comparison of either 5, 10, or 20 mg/kg of MDMA administered twice daily, eight hours apart, for ten consecutive days showed dose-related impairments of sequential learning and spatial learning and memory when the MDMA was administered in postnatal-days 11-20, but not 1-10 (Broening et al., 2001). Later research included a “drug-free holiday”, administering either 3, 10, or 30 mg/kg once a day for two five-day dosing periods, separated by a two-day drug-free holiday (Wiley et al., 2008). This resulted in an MDMA-induced increase of ambulatory activity in adolescents at doses of 10 and 30 mg/kg only, with adult rats being unaffected by the drug.

1.3.2 Sex

One of the reasons for often differing outcomes in research may be attributed to the sex of the animals used. Sex differences on various behavioral tests have been shown regardless of the presence of drugs. While females have been shown to have greater exploratory tendencies within the open-field (Archer, 1975), males have been shown to perform better on tests of spatial ability (Hughes, 2001).
When the effects of MDMA are examined according to the sex of the animal, the results are varied. This is largely due to the fact that the majority of studies have looked at males only (Faria et al., 2006; Gurtman et al., 2002; Maldonado & Navarro, 2000; Mayerhofer et al., 2001; Morley et al., 2001; O’Shea et al., 1998; Piper et al., 2005; Piper & Meyer, 2004; Reveron et al., 2005; Wiley et al., 2008). It is only recently that the effects of MDMA on each sex had been investigated (Broening et al., 2001; Koenig et al., 2005; Morley-Fletcher et al., 2002; Palenicek et al., 2005).

Some research indicates that female rats have greater sensitivity to the stimulatory effect of MDMA, with increased reactivity of serotonergic and dopaminergic systems considered to be caused by the effects of ovarian hormones (Palenicek et al., 2005). Other research indicates a greater resistance to MDMA among females, where an equal administration of MDMA resulted in the deaths of all the males, but only one third of the females (Koenig et al., 2005). MDMA appears to be more toxic in males than in females following repeated treatment (Easton & Marsden, 2006).

1.3.3 Age

Age is another factor that potentially has a tremendous effect on both acute and long-term effects of MDMA. Following early exposure to the drug coinciding with critical periods of known drug development, the ability of mice and rats to learn both spatial and path integration tasks, as well as anxiety levels, have altered and persisted into adulthood (Broening et al., 2001; Piper, 2007).

A number of researchers investigated the long-term effects of MDMA, ranging from 2-14 weeks following exposure to the drug (Broening et al., 2001; Gurtman et al., 2002; Mayerhofer et al.,
Research on neonatal exposure to the drug found significant impairments of sequential and spatial learning and memory still present in adulthood when the rats had been exposed to the drug at P11-20, but not if they had been exposed to the drug at P1-10 (Broening et al., 2001). Other research involving adolescent or adult animals provided conclusive evidence for a general decrease of 5-HT and 5-HIAA in the hypothalamus, amygdala, hippocampus, and striatum up to nine weeks following exposure to the drug (Gurtman et al., 2002; Mayerhofer et al., 2001; Morley-Fletcher et al., 2002). Further studies indicated increased anxiety following MDMA exposure as much as 12-14 weeks later (Morley et al., 2001). Collective research therefore indicates pervasive long-term biochemical and behavioral effects following MDMA exposure.

The adolescent period was investigated by a number of studies, the majority of which examined the effects of MDMA between four and ten days after exposure (Faria et al., 2006; Piper et al., 2005; Piper & Meyer, 2004). All found decreased attention and memory, as well as 5-HT content and serotonin transporter binding; however contrasting results were found for anxiety-like behaviours, though this could be due to the differences in the dosing regimens. Nevertheless, intermittent MDMA administration – whether over the course of one day, or every five days for a total of five days – was found to influence cognitive and affective functioning.

There exists a limited amount of research that directly compares periods of MDMA administration and the following long-term effects (Morley-Fletcher et al., 2002; Reveron et al., 2005; Wiley et al., 2008). A comparison of three different ages – P28, 38 and 52 – with testing at P80 indicated increased responsivity to the drug at middle and late adolescence, with a marked decrease of 5-HT concentration in the hypothalamus at adulthood (Morley-Fletcher et al., 2002). A comparison of different dosing regimens during adolescence, beginning at P28,
and tested at P70, found an increase in ambulatory activity in adolescents at doses of 10 and 30 mg/kg, although in adults, neither dose affected ambulatory activity (Wiley et al., 2008). MDMA administration during adolescence (P28) and adulthood (P70), when tested seven days following exposure to the drug, produced decreased levels of 5-HT only at P70, and a significantly greater reduction of the dopamine transporter, striatal dopamine, and DOPAC in adulthood than in adolescence (Reveron et al., 2005). The research indicates some contrasting evidence. Long-term studies show a general decrease of 5-HT with MDMA administration during adolescence. However other research suggests greater susceptibility of dopaminergic damage among adult animals, with the adolescents being less sensitive to the acute and stimulant effects of MDMA.

1.4 NEURAL DEVELOPMENT

Although neural development is most pronounced during childhood, it continues from the embryonic period right through adolescence. There are significant changes in cognitive, psychological, and social development, as well as age-specific alterations in behaviour and psychopharmacological responsivity (Laviola, Adriani, Terranova, & Gerra, 1999; Rice & Barone Jr., 2000). Throughout adolescence, a number of neurobiological and maturational changes occur, such as synaptic pruning, dendritic growth, system formation, and myelination (Montoya et al., 2002). Though there is an increase in gray matter volume, cortical interconnectivity, and the prefrontal cortex during childhood, this is followed by a decline to adult levels during adolescence (Rutter, 2007). This change within the central nervous system reflects the optimization of learning potential, accounted for through synaptic pruning and apoptosis (Chambers, Taylor, & Potenza, 2003; Greydanus & Patel, 2005). The glutamate
receptor $N$-methyl-$D$-aspartate (NMDA), which figures largely in synaptic plasticity and memory function, also goes through profound changes during adolescence. Both cortical binding to the receptors and levels of the receptor in the hippocampus peak during early adolescence, but decline to two thirds and one quarter of the levels respectively by adulthood (Spear, 2000). The growth and development of the central nervous system persists well beyond sexual maturity, and researchers have struggled to define the point at which development ends.

This period of development of the nervous system is considered especially vulnerable to environmental insults, due to the temporal and regional emergence of critical developmental processes, such as proliferation, migration, differentiation, synaptogenesis, myelination, and apoptosis (Rice & Barone Jr., 2000). Because the brain is changing during adolescence, it is more sensitive to the effects of certain drugs than the adult brain, and this can result in a variety of outcomes, such as greater drug intake, changes in neurotransmitter activity, and prevention of normal neurobiological development (Greydanus & Patel, 2005).

If exposure to a neurotoxicant such as MDMA occurs during the development of an organ, when it is more vulnerable to disruption, it may result in an increased rate of age-related decline in function (Rice & Barone Jr., 2000). As the brain is still undergoing crucial maturational changes, MDMA use during adolescence and subsequent depletion of 5-HT levels, in conjunction with altered catecholamines, may interfere with the developmental processes of the cerebral cortex, and potentially alter adult circuitry (Montoya et al., 2002).

The plasticity of the developing nervous system could also mask the long-term effects of a drug such as MDMA, and current research suggests adult rats are more sensitive to the long-term serotonin depletions following MDMA, whereas, at younger ages, they exhibit substantial and rapid neuroplasticity (Piper, 2007). Due to the plasticity of the developing brain, a significant
degree of serotonergic depletion may occur without any obvious impairments until the extent of
damage exceeds a certain threshold (Montoya et al., 2002). While the 5-HT system in adulthood
does have a considerable amount of neuroplasticity, the effects of MDMA may well be
particularly potent during development. Certainly it seems likely that depletion of 5-HT early in
life may result in the permanent destruction of serotonergic neurons (Montoya et al., 2002;
Piper, 2007). What appears to be a decrease in sensitivity to the drug could be the result of age-
dependent modulation of pharmacodynamics, such as a smaller number of neurotransmitter
receptors (Piper, 2007). The effects of MDMA on both learning and working memory, however,
seem to be independent of the developmental stage of exposure, with adults appearing more
sensitive to the long-term depletions of serotonin (Piper, 2007).

These developmental changes indicate that substance abuse in adolescence may have different,
and perhaps more substantial, effects than substance abuse in adulthood. These effects can range
from brain damage, changes in cognitive capabilities or electrophysiology, as well as possible
hormonal effects which may alter sexual maturation (Smith, 2003). In support of this, there are a
number of drugs which have different, and often longer-lasting effects in adolescents than in
adults. These can range from altered sensitivity to the same or a different drug at a later stage,
changes in adult cognitive capabilities, and damage to the central nervous system (Smith, 2003).
This would suggest that adolescent rats are less sensitive to the acute and repeated stimulant
effects of some, but not all of the drugs abused by humans in this age group (Wiley et al., 2008).
However, enough research implicates MDMA in the more sensitive category. This is supported
by evidence that forebrain structures which are essential for cognitive function, such as the
hippocampus and frontal cortex, are highly sensitive to MDMA. The serotonin system in these
regions undergoes dynamic and protracted development (Green et al., 2003). This would
suggest that age differentially modulates the biochemical and behavioural consequences of MDMA, as well as its long- and short-term effects (Piper, 2007). Collectively, animal findings indicate that substance use in early adolescence may not only disrupt normal pubertal development, but that it may also induce stronger effects on systems subserving plasticity and cognition than substance use in adulthood (Smith, 2003). This difference in effects of adolescent drug use when compared to adult drug usage can be attributed to the fact that the adolescent period is critical in terms of development and vulnerability to insults.

1.5 BEHAVIOURAL TESTS

A number of tests have been used in animal studies to measure drug effects. The purpose of the present study was to observe any long-term effects resulting from drug administration during early and late adolescence. The tests selected were largely chosen from those used in prior research involving the same drug, and are mainly exploration-based measures of emotionality or anxiety, and spatial or working memory (Belzung, 1999).

1.5.1 Open-Field Test

One of the most widely-used behavioural tests is the open-field, first introduced by Hall (1934), who interpreted defaecation as a sign of timidity. Since its introduction, the open-field test has varied in size and shape of the field, as well as some of the measures, but the principle has remained the same. Through various measures such as defaecation, locomotion, and rearing, the open-field test is commonly regarded as useful for assessing anxiety (Walsh & Cummins, 1976). This test and these observations are designed to measure both general activity and emotional reactivity (Prut & Belzung, 2003).
The measures used in the test include:

- **Defaecation** as a measure of emotionality or anxiety. An increase in numbers of faecal boluses indicates an increase in emotionality (Archer, 1975; Hall, 1934).

- **Ambulation**, or *transitions*, is a measure of activity and exploration, indicative of emotionality or anxiety. Greater exploration of the novel environment, demonstrated by ambulation, is indicative of lower anxiety. Ambulation in the present study was measured through the location of the animals in relation to the divisions (or squares) of the open-field, where a greater number of transitions from one division (or square) to another indicates more activity and exploration, and thus lower of emotionality (Hughes & Beveridge, 1987).

- **Centre** and *corner square occupancy* are measures of timidity or emotionality. They involve recording how frequently a rat occupies the four central squares, or the four corner squares. As rodents tend to prefer the periphery of the apparatus to the central area, an increase in centre square occupancy would indicate lower emotionality (Belzung, 1999; Prut & Belzung, 2003). Likewise, higher occupancy of the peripheral areas – either in corners or near walls – is used as an index of timidity (Walsh & Cummins, 1976) or emotionality (Prut & Belzung, 2003).

- **Rearing** is another measure of activity, which, combined with ambulation, has proved to reflect a stable individual trait – “nonspecific excitability level” (Walsh & Cummins, 1976). A decrease in rearing or vertical exploration indicates increased emotionality or anxiety.

- **Grooming** is considered to be a specific rodent behavioural response to stressful situations, whereby an increase of grooming in a novel environment is indicative of increased
emotionality or anxiety (Moody, Merali, & Crawley, 1988). Furthermore, grooming has been shown to be negatively related to indexes of high-activity states, with greater activity resulting in less grooming (Walsh & Cummins, 1976).

This test has been popular in the area of adolescence and drug use (Maldonado & Navarro, 2000; Palenicek et al., 2005; Piper & Meyer, 2004). Following on from prior research, we would expect that animals treated with MDMA would show greater emotional reactivity than control animals. Furthermore, due to ongoing neural development and resulting vulnerability to insults, animals treated during early adolescence would be expected to show higher subsequent emotionality or anxiety than animals treated during late adolescence.

1.5.2 Light/Dark Choice

The light-dark test was developed in the 1980s as a model of anxiety based around the conflict between the aversion of rodents to large and brightly lit areas and the tendency to explore novel environments (Crawley & Goodwin, 1980). The tendency to remain in the dark box is the norm among rats, with the novel environment reacted to as aversive and frightening. Anti-anxiety drugs act in suppressing this aversion, and increasing exploratory activity – such as between a brightly lit arena and a dark enclosed compartment (Crawley & Davis, 1982). Higher emotionality, or anxiety, is indicated by the tendency to avoid the light side (Hascoët, Bourin, & Dhonnchadha, 2001).

The measures used in the test include:

- **Transitions** between the light and dark areas as an index of emotionality, with more transitions indicating more exploratory behaviour (Belzung, 1999; Misslin, Belzung, &
Vogel, 1989). Thus, more exploration indicates lower emotionality or anxiety (Bourin & Hascoët, 2003; Crawley & Davis, 1982).

- *Time spent in light*, where longer times in the dark areas are regarded as reflecting lower levels of anxiety in rats. So, more time spent in the light indicates less emotional reactivity.

Although the light-dark test has at times resulted in contradictory outcomes (largely because of a lack of adequate test standardization) (Hascoët & Bourin, 1998), prior research in the area of adolescence and drug use has found a decrease in exploratory activity, though time spent in the light remained unaffected (Maldonado & Navarro, 2000). We would expect similar results, with a decrease in exploratory activity among drug-treated animals, in particular amongst animals treated at a younger age.

1.5.3 *Responsiveness to Brightness Test*

This test is a derivative of a test of neotic preference designed in the 1950s (Dember, 1956). When one arm of a Y maze has changed in brightness from what it was during an earlier trial, rats have been shown to explore this novel arm first (Hughes & Maginnity, 2007). It is a test of short-term spatial memory, as the rats must be able to identify which arm has changed by remembering the colour and position of both arms (Hughes & Maginnity, 2007).

The measures used in the test include:

- *Time in novel arm* as a percentage, where greater time spent in the novel arm would indicate greater novelty preference.
• *Entries of novel arm* as a percentage, which is seen as a measure of short-term recognition memory (Hughes, 2001). More entries of and times spent in the novel arm would indicate better short-term spatial memory.

• *Total time spent in both arms* as a measure of exploration, where longer time spent in both arms indicates more exploration and less emotionality or anxiety.

• *Total entries of both arms* as a measure of emotionality, where more entries to both arms are indicative of greater activity and lower emotional reactivity, or anxiety.

Prior research has found that females appear to be less responsive to change than males as determined by entries of and time spent in the changed arm (Hughes, 2001). Thus similar results would be expected for the female animals in general. These effects may be further modified through MDMA administration, with the drug-treated rats expected to show a reduced responsiveness to brightness change, and in particular the younger animals due to the continuing neurodevelopment.

**1.5.4 Object Recognition Test**

The process of habituation to novelty can be seen as a form of learning. The object recognition test is a measure of working memory involving knowledge of previously encountered objects. The preference for the novel object measures working memory in rats (Blanchard, Shelton, & Blanchard, 1970; Ennaceur & Delacour, 1988; Sutcliffe, Marshall, & Neill, 2007).

The test comprises a test trial and a follow-up trial, during which the frequency and duration of exploration of each object is recorded. This enables the calculation of a discrimination index.
from exploration of the novel object compared to the familiar object. The discrimination index is thus a measure of novelty preference, and working memory.

MDMA administration has been shown to influence behaviour in this task when a 15-minute interval is provided between the two trials (Morley et al., 2001). Prior research has found a general decrease in object recognition memory following MDMA administration (Meyer et al., 2008; Morley et al., 2001; Piper & Meyer, 2004). Similar results would be expected, with a general decrease in object recognition memory among drug-treated animals, and also the expectation that younger animals would be more affected than older animals due to their lesser degree of neurodevelopment.

1.6 RATIONALE FOR CURRENT STUDY

A significant amount of research indicates that the use of the recreational drug MDMA, or “Ecstasy” can lead to detrimental effects in memory and behaviours such as anxiety. There is also some indication that there may be developmental effects associated with the use of this drug, depending on the timing of exposure to the drug. Despite this, there has been limited research focused on the effects of drug use specifically during adolescence, and in particular the long-term implications. This research aims to assess these developmental differences more concretely, with a direct comparison of what the results are like in adulthood depending on whether MDMA is administered in early adolescence or young adulthood.

2.0 Aims and Hypotheses of this Study
The aim of this experiment was to discover any age-related developmental differences in the subsequent effects of MDMA. There were two experimental groups, one defined as the early adolescent period, and the other as late adolescence. In rats, these developmental periods correspond to postnatal days (P) 35+ after birth for early, and P45+ for late adolescence.

Based on prior research, it was expected that the younger the animals were at the age of MDMA administration, the more severe would be the long-term effects in adulthood.

3.0 Methods

3.1 SUBJECTS

The subjects were 40 male and 40 female PVG/C hooded rats from the breeding colony at the University of Canterbury. The rats were caged in groups of 3-4 individuals of the same sex from different litters with free access to food (commercial rat pellets) and drinking water. They were kept in an ambient temperature of 22°C on a 12 hour light/dark cycle (with lights on at 8am).

3.2 TREATMENT

At the beginning of treatment, half of the rats were 35 days old, and approaching puberty: the periadolescent or early adolescent period (Spear & Brake, 1983). The other half were 45 days old, and entering the developmental equivalent of late adolescence, or young adulthood.

Half of the rats within each age group were intraperitoneally injected each day with a saline control solution (1 ml/kg) for a period of ten days. The rest received a 10 mg/kg injection of MDMA of equivalent volume to control subjects.
Each rat was weighed every second day of treatment to enable an accurate dosage. These weights were recorded to observe any effect on weight gain which may be accounted for by MDMA. All rats were also later weighed at PND 90 to record any present long-term effects on weight.

The dose chosen has been consistently shown to have an effect on 5-HT levels and cognitive impairments. The treatment regimen was selected to demonstrate the effects of consistent, chronic and repeated drug use, following on from prior research which has focused on intermittent drug exposure (Meyer et al., 2008; Morley-Fletcher et al., 2002).

Thus, the number of rats in each group were:

10 early adolescent control males
10 early adolescent control females
10 early adolescent MDMA males
10 early adolescent MDMA females
10 late adolescent control males
10 late adolescent control females
10 late adolescent MDMA males
10 late adolescent MDMA females

The numbers of rats were chosen on the basis of the minimum numbers required to demonstrate effects that take account of possible differences in the way males and females might react to the drug.
3.3 APPARATUS

3.3.1 Open-Field

The apparatus consisted of a wooden 600x600 mm open-field, (60x60 cm) with Perspex walls. The floor was painted black, and divided by white lines into a 3x3 grid of nine numbered squares 15x15 cm.

3.3.2 Light-Dark Box

The light-dark apparatus was constructed from wood and comprised a 200x150x200 mm-high unlit start box painted black, with a hinged wooden lid. This opened via a sliding guillotine door into a 500x400x200 mm-high unpainted arena with a hinged clear Perspex lid, and was illuminated from above.

3.3.3 Y-Maze

The apparatus comprised an enclosed, unpainted wooden Y-maze that was 10cm wide and 14cm high, and consisted of a 30cm stem and two 45cm long arms with an angle of 120° between them. The stem was split in half by a guillotine door, enabling the animal to be placed into the “start box”. A transparent Perspex hinged lid covered the maze, finishing at the guillotine door. Each arm contained a removable black or white aluminum insert, which occupied the width, height, and 40cm of the length of the arms.
3.4 BEHAVIOURAL TESTING

Each rat was tested at two different periods: immediately following saline or MDMA administration on the tenth day of treatment, and at PND 90. Observation of acute effects of MDMA in the open-field was carried out on the final day of treatment to confirm effectiveness of the drug.

Long-term effects of MDMA were observed once the rats were 90 days old, and subjected to the following behavioral tests: open-field test, light-dark preference, responsiveness to brightness change, and object recognition. Neither of these tests require training, and are purely observational.

3.4.1 Acute Effects

Shortly after administration of either saline or MDMA on day ten of the treatment, each rat was placed in the centre of the open-field, and its behaviours observed at an interval of every five seconds for three minutes. The responses recorded included rearing up on hind legs, grooming and walking. Ambulation was calculated by counting the number of times each rat was located in a different square from where it had been five seconds earlier. Number of faecal boluses left in the apparatus at the end of the trial were also counted, and following each trial any faeces were removed and the apparatus was sprayed with a cleaning solution and wiped down with a paper towel.

3.4.2 Open-Field Test

Similar to the process described above, in the open-field test carried out at PND 90, each rat was placed in the centre of the open-field and its location and behaviour recorded every three
seconds for five minutes. The duration of the trial was both longer and more frequent than what had been observed for measuring acute effects, in order to obtain more reliable data. The responses recorded included rearing up on hind legs and grooming. Ambulation, or locomotor activity, was later calculated by counting the number of times each rat was located in a different square from where it had been three seconds previously. Numbers of faecal boluses left in the apparatus at the end of the trial were also counted. Following each trial, any faeces were removed, and the apparatus was sprayed with a cleaning solution and wiped down with a paper towel.

3.4.3 Light-Dark Preference Test

In the light-dark preference test, each rat was placed in the dark compartment of the light-dark box for 30 seconds, with the slide separating the compartments in place. The slide was then withdrawn, and the rat allowed free access to both compartments for five minutes, with the total time spent in the light side, and the entries of it, being recorded using a computer program. Following each trial, any faeces were removed, and the apparatus was sprayed with a cleaning solution and wiped down with a paper towel.

3.4.4 Responsiveness to Brightness Change Test

In the responsiveness to brightness change test, each rat was placed in the stem of the Y maze, with one arm containing a black insert, and the other a white insert. After a six-minute acquisition trial, the rat was removed and the white and black inserts replaced by two clean black ones. The rat was then put back into the stem for a three-minute retention trial. During the test, the total number of entries of each arm, as well as the total time spent in each arm was recorded. Each rat experienced two acquisition/retention trials, with several days in between.
The white arm was on the left for one acquisition trial, and on the right for the other. From the total entries of and time spent in each arm, it was later possible to calculate the percentage of entries of and time spent in the changed (or novel) arm. Following each trial, any faeces were removed, and the apparatus was sprayed with a cleaning solution and wiped down with a paper towel.

3.4.5 Object Recognition Test

In the object recognition test, two identical objects (small clear glass jars around 5cm high and across, or tall clear glass jars around 8cm high and 3cm across) were placed in two diagonally opposite corners (bottom left and top right, or top left and bottom right) of a square Perspex open-field, 10cm from the walls. The animals were then placed in the centre of the open-field described above, and three minutes later, the rat was removed from the apparatus. One of the familiar objects was then replaced by a novel object, and the rat was placed back in the open-field 15 minutes later for the three-minute test trial. During the test trial, the frequency and duration of exploration of each object was recorded. Every three seconds the location of the animal was recorded, and exploration was defined as physical proximity to the object. This was measured by the frequency of occupancy of the square that each object was in.

The discrimination index was later calculated by subtracting exploration of the familiar object from exploration of the novel object, and dividing the result by total exploration time. The location of the novel object was alternated between trials to control for a corner preference, as well as the novel object itself. Each object was cleaned by being wiped down with a paper towel which had been sprayed with a disinfectant solution to remove any familiar olfactory cues before being placed in the open-field. Following each trial, any faeces were removed, and the apparatus was sprayed with a cleaning solution and wiped dry with a paper towel.
4.0 Statistical Analyses

All responses recorded in each type of apparatus were subjected to separate 2 (drug group) x 2 (treatment period) x 2 (sex) ANOVAs. If any significant interactions occurred, post hoc comparisons were made with Newman-Keuls tests (p <0.05).
5.0 Results

5.1 BODY WEIGHTS
Table 1: Mean (± S.E.M) percent (%) of weight increase P35 to PND 43 for male and female rats that were treated with either saline or MDMA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$F(1,36)$</th>
<th>Sex</th>
<th>$F(1,36)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td></td>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>MDMA</td>
<td></td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>Weight increase</td>
<td>24.35 (±0.35)</td>
<td>21.60 (±0.25)</td>
<td>$4.48^*$</td>
</tr>
<tr>
<td></td>
<td>27.70 (±0.29)</td>
<td>18.25 (±0.11)</td>
<td>$16.73^{**}$</td>
</tr>
</tbody>
</table>

* $P<0.05$; ** $P<0.01$.

Table 2: Mean (± S.E.M) percent (%) of weight increase P45 to PND 53 for male and female rats that were treated with either saline or MDMA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$F(1,36)$</th>
<th>Sex</th>
<th>$F(1,36)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td></td>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>MDMA</td>
<td></td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>Weight increase</td>
<td>21.80 (±1.16)</td>
<td>18.89 (±1.45)</td>
<td>$9.29^*$</td>
</tr>
<tr>
<td></td>
<td>24.54 (±0.81)</td>
<td>15.55 (±0.98)</td>
<td>$60.98^{**}$</td>
</tr>
</tbody>
</table>

* $P<0.05$; ** $P<0.01$. 
5.1.1 Body Weights of Early and Late Adolescents

As shown in Figure 1, the weight of rats treated during early adolescence increased steadily with each repeated measure ($F[4,144] = 689.57$, $p = .0001$). However, there was a significant sex x post natal design interaction for weight ($F[4,144] = 31$, $p = .0001$), possibly accounted for by a more rapid increase for males in overall weight gain.

Male rats were significantly heavier than female rats ($F[1,36] = 56.08$, $p = .0001$).

![Figure 1: Body weight increase of male & female early adolescents](image)

For animals treated during late adolescence, there was a significant group x post natal design interaction ($F[4,144] = 4.44$, $p = .0021$) seen in Figure 2, where the weight of the rats increased steadily with each repeated measure ($F[4,144] = 651.53$, $p = .0001$). This was most likely accounted for by slightly different rates of increase for the particular groups and
sexes involved. However, as shown in Figure 3, there was also a significant sex x post natal design interaction for weight ($F[4,144] = 82.69, p = .0001$), possibly accounted for by a more rapid increase for males in overall weight gain.

Male rats were significantly heavier than female rats ($F[1,36] = 189.28, p = .0001$).

**Figure 2: Body weight increase of late adolescents**
5.1.2 Percentage of Weight Increase

As shown in Table 2, MDMA treatment significantly reduced the percentage of weight increase among early adolescents from the first day of treatment (P35) to P43, where the MDMA group overall had a significantly smaller percent weight increase than the control group. However, a significant interaction between MDMA and sex revealed that this smaller weight increase only applied to males (see Figure 4).

Male rats had a significantly greater percent of weight increase than female rats.
* significantly different from saline group (p < 0.05) for that particular sex

**Figure 4: Percentage of weight increase of early adolescents (P35-43)**

As shown in Table 2, among rats treated during late adolescence, the percentage of weight increase was unaffected by MDMA treatment. As expected, there was a significant difference depending on the sex of the rats ($F[1,36] = 60.98, p = .0001$), where males experienced greater weight increase percent than females.

**5.1.3 Effects on Body Weight at PND 90**

Significant main effects were found for MDMA treatment for post-testing body weight, recorded to observe the presence of any present long-term effects on weight ($F[1, 72] = 14.7, p = .0003$), for the treatment period ($F[1,72] = 4.94, p = .0294$), and for sex of the rats ($F[1,72] = 1136.35, p = .0001$).
There was also a significant treatment period x MDMA interaction for body weight following testing ($F[1,72] = 14.26, p = .0003$). However, all of the above results must be considered in the light of a significant treatment period x sex x MDMA interaction for post-testing body weight ($F[1,72] = 14.26, p = .0003$). As outlined in Figure 5, overall, males weighed significantly more than females. Male rats treated with MDMA weighed more than controls only when treated during late adolescence, whereas female rats treated with MDMA weighed more than controls only when treated during early adolescence. Male rats treated with saline during late adolescence weighed less in adulthood than those that had been treated during early adolescence.

* significantly different ($p < 0.05$) from saline for that particular sex

*a significantly different ($p < 0.05$) from early adolescent treatment period for that particular sex

**Figure 5: Post-testing body weights**
5.2 ACUTE EFFECTS OF MDMA ON OPEN-FIELD BEHAVIOUR

5.2.1 Early Adolescents

As seen in Table 3, significant MDMA treatment effects or interactions involving MDMA occurred for the rearing, grooming, and walking measures of the open-field. This provided evidence that the dose chosen was behaviourally effective. However, there appeared to be no significant differences between male and female animals, suggesting that MDMA treatment in early adolescence resulted in acute behavioural effects independent of sex.

Significant results were found for the main MDMA treatment effect for rearing, where the MDMA group showed less rearing behaviour than the control group.

The main MDMA treatment effect for grooming was shown to be significant, where the MDMA group showed less grooming behaviour than the control.

Results were significant for the main MDMA treatment effect for walking, where the MDMA group walked more than the control.
Table 3: Mean (± S.E.M) 5-s acute observations of open-field ambulation, rearing, grooming, walking and defaecation for male and female rats that were treated with either saline or MDMA during early adolescence

<table>
<thead>
<tr>
<th>Treatment</th>
<th>F(1,36)</th>
<th>Sex</th>
<th>F(1,36)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>MDMA</td>
<td>Male</td>
</tr>
<tr>
<td>Ambulation</td>
<td>45.65 (±2.10)</td>
<td>44.90 (±2.98)</td>
<td>0.04</td>
</tr>
<tr>
<td>Rearing</td>
<td>19.55 (±1.36)</td>
<td>2.65 (±0.96)</td>
<td>98.58***</td>
</tr>
<tr>
<td>Grooming</td>
<td>1.50 (±0.18)</td>
<td>0.50 (±0.26)</td>
<td>10.4*</td>
</tr>
<tr>
<td>Walking</td>
<td>19.80 (±0.77)</td>
<td>28.15 (±2.01)</td>
<td>15.42**</td>
</tr>
<tr>
<td>Faecal boluses</td>
<td>1.65 (±0.54)</td>
<td>0.65 (±0.34)</td>
<td>2.46</td>
</tr>
</tbody>
</table>

* P <0.05; ** P <0.01; *** P <0.001.
5.2.2 Late Adolescents

As seen in Table 4, significant MDMA treatment effects or interactions involving MDMA occurred also for the rearing, grooming, and walking measures of the open-field. This provided further evidence that the dose chosen was behaviourally effective.

The results displayed a significant difference between male and female animals on the grooming measure, where female rats showed more grooming behaviours than male rats.

Significant results were found for the main MDMA treatment effect for rearing, where the MDMA group showed less rearing behaviour than the control group.

The main MDMA treatment effect for grooming was shown to be significant, where the MDMA group showed less grooming behaviour than the control.

Results were significant for the main MDMA treatment effect for walking, where the MDMA group walked more than the control.
Table 4: Mean (± S.E.M) 5-s acute observations of open-field ambulation, rearing, grooming, walking and defaecation for male and female rats that were treated with either saline or MDMA during late adolescence

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
<th>$F(1,36)$</th>
<th>Sex</th>
<th>$F(1,36)$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>MDMA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambulation</td>
<td>47.35 (±1.08)</td>
<td>43.65 (±2.76)</td>
<td>1.48</td>
<td></td>
</tr>
<tr>
<td>Rearing</td>
<td>30.60 (±1.07)</td>
<td>7.55 (±2.27)</td>
<td>82.88***</td>
<td></td>
</tr>
<tr>
<td>Grooming</td>
<td>1.95 (±0.44)</td>
<td>0.10 (±0.10)</td>
<td>19.22***</td>
<td></td>
</tr>
<tr>
<td>Walking</td>
<td>15.00 (±1.09)</td>
<td>34.70 (±2.30)</td>
<td>13.99***</td>
<td></td>
</tr>
<tr>
<td>Faecal boluses</td>
<td>0.50 (±0.34)</td>
<td>0.60 (±0.23)</td>
<td>0.66</td>
<td></td>
</tr>
</tbody>
</table>

* $P <0.05$; ** $P <0.01$; *** $P <0.001$. 
5.3 LONG TERM EFFECTS OF MDMA

5.3.1 Open-Field

As can be seen in Table 5, significant MDMA treatment effects or interactions involving MDMA occurred for all of the open-field measures apart from centre square occupancy and rearing.
Table 5: Mean (± S.E.M) 3-s observations of open-field ambulation, centre occupancy, corner occupancy, rearing, grooming and defaecation for male and female rats that were treated with either saline or MDMA during either early or late adolescence

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
<th>F(1,72)</th>
<th>Adolescent period</th>
<th>F(1,72)</th>
<th>Sex</th>
<th>F(1,72)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>MDMA</td>
<td>Early</td>
<td>Late</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Ambulation^a</td>
<td>55.95 (±1.87)</td>
<td>55.22 (±1.92)</td>
<td>0.09</td>
<td>54.70 (±2.13)</td>
<td>56.47 (±1.62)</td>
<td>0.54</td>
</tr>
<tr>
<td>Centre occupancy</td>
<td>8.40 (±0.65)</td>
<td>10.02 (±0.99)</td>
<td>1.78</td>
<td>9.20 (±1.02)</td>
<td>9.23 (±0.64)</td>
<td>0.01</td>
</tr>
<tr>
<td>Corner occupancy</td>
<td>52.17 (±1.96)</td>
<td>43.05 (±1.53)</td>
<td>13.09***</td>
<td>47.92 (±2.27)</td>
<td>47.30 (±1.45)</td>
<td>0.06</td>
</tr>
<tr>
<td>Rearing</td>
<td>25.25 (±1.38)</td>
<td>24.10 (±1.30)</td>
<td>0.39</td>
<td>23.40 (±1.42)</td>
<td>25.95 (±1.24)</td>
<td>2.55</td>
</tr>
<tr>
<td>Grooming^b</td>
<td>1.35 (±0.27)</td>
<td>1.40 (±0.29)</td>
<td>0.02</td>
<td>1.62 (±0.31)</td>
<td>1.12 (±0.24)</td>
<td>1.75</td>
</tr>
<tr>
<td>Fecal boluses</td>
<td>0.20 (±0.11)</td>
<td>0.70 (±0.23)</td>
<td>4.14*</td>
<td>0.43 (±0.18)</td>
<td>0.47 (±0.20)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

^a Treatment x adolescent period interaction significant (see text).

^b Treatment x sex interaction significant (see text).

* P <0.05; ** P <0.01; *** P <0.001.
5.3.1.1 Ambulation

There was a significant treatment period x MDMA interaction for ambulation ($F[1,72] = 13.3, p = .0005$). As outlined in Figure 6, while there was no significant difference between control and MDMA rats treated during early adolescence, MDMA led to significantly less ambulation than controls for rats treated during late adolescence.

As shown in Table 5, female rats displayed significantly more ambulation than males.

* Significantly different from saline for that particular treatment age

* Significantly different from early adolescence saline group

**Figure 6: Open-field ambulation: treatment period x MDMA interaction**
5.3.1.2 Corner Occupancy

Significant results were found for the main MDMA treatment effect on corner square occupancy, where the MDMA group occupied fewer corners than the control group.

5.3.1.3 Grooming

There was a significant MDMA x sex interaction for grooming \((F[1,72] = 5.06, p = .0276)\) which can be accounted for by MDMA (but not control) females showing more grooming behaviour than males (MDMA male mean ± SEM = .8 ± .05, control males = 1.6 ± .08, MDMA females = 2.0 ± .11, control females = 1.1 ± .09, \(F[1,72] = 5.06, p = .0276\)).

5.3.1.4 Defaecation

Significant results were found for the main MDMA treatment effect on faecal boluses, where the MDMA group defaecated slightly more than the control.

As shown in Table 5, male rats defaecated more than females.

5.3.2 Light/Dark Choice

The results for behaviour in the light-dark box are outlined in Table 6.
Table 6: Mean (± S.E.M.) time(s) spent in the light side of the light-dark box and transitions between the two halves for male and female rats that were treated with either saline or MDMA during either early or late adolescence.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>F(1,72) Adolescent period</th>
<th>F(1,72) Sex</th>
<th>F(1,72)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>Early</td>
<td>Late</td>
<td>Male</td>
</tr>
<tr>
<td>MDMA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entries of light side&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.27 (±0.60)</td>
<td>10.88 (±0.52)</td>
<td><strong>9.39</strong>&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Time in light side&lt;sup&gt;b&lt;/sup&gt;</td>
<td>116.16 (±7.15)</td>
<td>113.93 (±7.35)</td>
<td><strong>0.05</strong></td>
</tr>
</tbody>
</table>

<sup>a</sup> Treatment x adolescent period interaction significant (see text).

<sup>b</sup> Adolescent period x sex interaction significant (see text).

* P <0.01
5.3.2.1 Time Spent in Light Side

There was a significant treatment period x sex interaction for time spent in light \( (F[1,72] = 4.17, p = .0448) \). As outlined in Figure 7, there was a significant increase for time spent in light for females treated during late adolescence compared to early adolescence. Late adolescent females spent more time in light than early adolescent females.

* significantly different \((p < 0.05)\) from early adolescence for that particular sex

**Figure 7: Light/dark time in light: treatment period x sex interaction**

5.3.2.2 Entries of Light Side

A significant treatment group x MDMA interaction \( (F[1,72] = 4.17, p = .0447) \), revealed that, rats treated with MDMA during late adolescence entered the light side less often than those
treated with saline (see Figure 8). However there was no significant effect of MDMA in rats treated during early adolescence.

* significantly different from saline for that particular treatment age

Figure 8: Light/dark transitions: treatment period x MDMA interaction

5.3.3 Responsiveness to Brightness Change Test

As can be seen in Table 7, significant main effects occurred for time spent in both arms, entries of both arms, but not percentage of time in the novel arm or percentage of entries of novel arm.
Table 7: Mean (± S.E.M.) percent (%) entries of and time spent in the novel arm, and entries of and time(s) spent in both arms of the Y maze for both male and female rats that were treated with either saline or MDMA during either early or late adolescence.

| Treatment  | F(1,72) Adolescent period | F(1,72) Sex | F(1,72) |  |
|------------|---------------------------|-------------|---------|  |
|            | Saline  | MDMA        | Early   | Late   | Male | Female |  |
| Entries of both arms\(^a\) | 9.93 (±0.62) | 7.90 (±0.65) | 6.56\(^*\) | 7.45 (±0.56) | 10.38 (±0.66) | 13.68\(^{***}\) | 8.12 (±0.72) | 9.70 (±0.55) | 3.97\(^*\) |
| Time in both arms (s) | 143.65 (±7.05) | 113.94 (±9.32) | 7.52\(^{**}\) | 128.41 (±8.73) | 129.18 (±7.63) | 0.01 | 117.91 (±8.94) | 139.69 (±6.96) | 3.99\(^*\) |
| % novel arm entries | 55.40 (±1.86) | 55.98 (±2.02) | 0.06 | 54.88 (±2.12) | 56.52 (±1.72) | 0.37 | 57.42 (±2.37) | 54.00 (±1.36) | 1.54 |
| % time in novel arm | 51.97 (±2.63) | 53.26 (±3.05) | 0.11 | 50.35 (±3.53) | 54.92 (±1.80) | 1.23 | 52.59 (±3.34) | 52.62 (±2.29) | 0.66 |

\(^a\) Treatment x adolescent period interaction significant (see text).

\(^*\) P < 0.05; \(^{**}\) P < 0.01; \(^{***}\) P < 0.001.
5.3.1 Entries of Both Arms

There was a significant main effect of MDMA treatment on entries of both arms. However, there was also a significant treatment period x MDMA interaction for this measure ($F_{1,72} = 5.33, p = .0239$). As outlined in Figure 9, both early adolescent groups made fewer entries than the late adolescent groups. Control (but not MDMA) rats treated during late adolescence made significantly more entries of both arms than rats treated during early adolescence. The MDMA effect was significant only for those treated in late adolescence.

As shown in Table 7, males made fewer entries than females.

5.3.2 Time in Both Arms

As shown in Table 7, the MDMA group spent less time in both arms than the control, and males spent significantly less time in both arms than females.
* Significantly different from saline for that particular treatment age

a Significantly different from early adolescent saline group

Figure 9: Responsiveness to brightness change: entries of both arms: treatment period x MDMA interaction

5.3.4 Object Recognition Test

As shown in Table 8, no significant effects occurred for the discrimination ratio measure of the novel object recognition test.
Table 8: Mean (± S.E.M.) discrimination ratio of the object recognition test for both male and female rats that were treated with either saline or MDMA during either early or late adolescence.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$F(1,72)$ Adolescent period</th>
<th>$F(1,72)$ Sex</th>
<th>$F(1,72)$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early</td>
<td>Late</td>
<td>Male</td>
</tr>
<tr>
<td>Saline</td>
<td>0.56 (±0.00)</td>
<td>0.56 (±0.00)</td>
<td>0.57 (±0.00)</td>
</tr>
<tr>
<td>MDMA</td>
<td>0.57 (±0.00)</td>
<td>0.58 (±0.00)</td>
<td>0.57 (±0.00)</td>
</tr>
</tbody>
</table>

Discrimination ratio

The table shows that the discrimination ratio for male rats was slightly higher than for female rats, with a significant difference in the early adolescent period (0.56 ± 0.00 vs. 0.57 ± 0.00, $F(1,72) = 0.07, p < 0.05$). There was also a slight but non-significant difference in the late adolescent period (0.58 ± 0.00 vs. 0.57 ± 0.00). The treatment with MDMA did not significantly affect the discrimination ratio compared to saline treatment in both male and female rats.
### 5.4 SUMMARY OF RESULTS

#### 5.4.1 MDMA Treatment

Table 9: Summary of the subsequent behavioral effects of MDMA treatment during adolescence

<table>
<thead>
<tr>
<th>Apparatus and measure</th>
<th>Behavioral effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Open-field:</strong></td>
<td></td>
</tr>
<tr>
<td>Ambulation</td>
<td>Decreased only for rats treated during late adolescence</td>
</tr>
<tr>
<td>Rearing</td>
<td>No effect</td>
</tr>
<tr>
<td>Grooming</td>
<td>No effect</td>
</tr>
<tr>
<td>Occupancy of centre squares</td>
<td>No effect</td>
</tr>
<tr>
<td>Occupancy of corner squares</td>
<td>Decreased</td>
</tr>
<tr>
<td>Faecal boluses</td>
<td>Increased</td>
</tr>
<tr>
<td><strong>Light-dark box:</strong></td>
<td></td>
</tr>
<tr>
<td>Time in the light side</td>
<td>No effect</td>
</tr>
<tr>
<td>Entries of the light side</td>
<td>Decreased only for rats treated during late adolescence</td>
</tr>
<tr>
<td><strong>Y maze:</strong></td>
<td></td>
</tr>
<tr>
<td>% entries of the novel arm</td>
<td>No effect</td>
</tr>
<tr>
<td>% time in the novel arm</td>
<td>No effect</td>
</tr>
<tr>
<td>Total entries/day of both arms</td>
<td>Decreased only for rats treated during late adolescence</td>
</tr>
<tr>
<td>Total time spent/day in both arms</td>
<td>Decreased</td>
</tr>
</tbody>
</table>

NOTE: The correlation between these latter measures was significant i.e., r(78) = 0.71, p<0.001.

**Object recognition:** No effect
### 5.4.2 Sex Differences

Table 10: Summary of the subsequent behavioral effects of sex

<table>
<thead>
<tr>
<th>Apparatus and measure</th>
<th>Behavioral effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Open-field:</strong></td>
<td></td>
</tr>
<tr>
<td>Ambulation</td>
<td>Less for males</td>
</tr>
<tr>
<td>Rearing</td>
<td>No effect</td>
</tr>
<tr>
<td>Grooming</td>
<td>Less for MDMA-treated males</td>
</tr>
<tr>
<td>Occupancy of centre squares</td>
<td>No effect</td>
</tr>
<tr>
<td>Occupancy of corner squares</td>
<td>No effect</td>
</tr>
<tr>
<td>Faecal boluses</td>
<td>More for males</td>
</tr>
<tr>
<td><strong>Light-dark box:</strong></td>
<td></td>
</tr>
<tr>
<td>Time in the light side</td>
<td>More for females but only for late adolescent treated rats</td>
</tr>
<tr>
<td>Entries of the light side</td>
<td>No effect</td>
</tr>
<tr>
<td><strong>Y maze:</strong></td>
<td></td>
</tr>
<tr>
<td>% entries of the novel arm</td>
<td>No effect</td>
</tr>
<tr>
<td>% time in the novel arm</td>
<td>No effect</td>
</tr>
<tr>
<td>Total entries/day of both arms</td>
<td>Less for males</td>
</tr>
<tr>
<td>Total time spent/day in both arms</td>
<td>Less for males</td>
</tr>
<tr>
<td><strong>Object recognition:</strong></td>
<td>No effect</td>
</tr>
</tbody>
</table>
6.0 Discussion of Results

6.1 GENERAL DISCUSSION

MDMA exposure during adolescence was shown to have a number of significant long-term effects, and this was often dependent on whether drug administration occurred during early or late adolescence.

Animals that had been exposed to MDMA during late, but not during early adolescence, showed an increase in emotional reactivity, or anxiety. This was seen through various measures in the open-field test, the light-dark box, and the responsiveness to brightness change test.

When sex of the animal was also considered, differences between the sexes in responsiveness to MDMA, in addition to differences between the ages, were made apparent. Consistent with prior research, males appeared to be more emotionally reactive or anxious than females regardless of the age of drug administration (Archer, 1975; Palenicek et al., 2005). There appeared to be higher emotionality or anxiety in males than in females as reflected in several open-field and responsiveness to brightness measures.

For animals that had been treated with MDMA during late adolescence, there appeared to be an increase in emotionality, but only for females. This finding was contrary to prior research, which had found no sex differences (Maldonado & Navarro, 2000).

MDMA was shown to influence emotionality mainly when it was administered during late adolescence, where there was a significant increase of anxiety. Overall, however, males were shown to be more emotionally reactive to MDMA than females, with greater emotionality or anxiety in males and lower emotionality in females.
6.1.1 Acute Effects of MDMA

MDMA administration in adolescence showed significant acute behavioural effects, indicating the effect of the drug treatment. Open-field data shortly after administration on the last day of treatment showed that both early and late adolescent rats displayed significantly less rearing and grooming behaviours and more walking behaviours, accounted for by MDMA. Rats which had been treated with the drug indicated an increase of anxiety as seen through decreased rearing. While the increase in ambulation and the decrease in grooming behaviours is normally indicative of a decrease in anxiety, the properties of MDMA are such that it stimulates motor activity in animals. Furthermore, as grooming is negatively related to indexes of high-activity states (Walsh & Cummins, 1976), the data is consistent with the concept of MDMA-treated rats showing less grooming behaviour due to greater ambulatory activity. The same explanation could be given for the decrease of rearing behaviour among MDMA-treated animals: rather than only being accounted for by an increase of anxiety, it is possible that rearing, too, is lessened due to greater ambulation.

Sex effects were seen only among late-adolescent animals, where female rats treated with MDMA showed significantly more grooming behaviours than male rats. MDMA treatment in early adolescence appeared to have no significant effect between male and female rats.

This shows that MDMA had acute, significant effects on the animals treated with it compared to the controls. In other words, it was clear that the dose used in this study was behaviourally effective.
6.1.2 Body Weights

As expected, adolescent animals went through significant weight gain, witnessed daily, with males being generally heavier than females.

MDMA treatment during the early adolescent developmental period showed significant differences from the first day of treatment. From post-natal day 35 to P43, the percentage of weight increase was significantly smaller among rats treated with MDMA. This is in support of prior research showing that MDMA significantly reduces the rate of growth which is most likely due effects of the drug on physiological processes which are related to factors that may influence weight gain, such as reduced food and water intake, and increased urination and defaecation (Piper, 2007; Piper & Meyer, 2004).

However rats treated during late adolescence appeared unaffected by MDMA, with weight gain increasing over time, and more so for male than female rats. The drug did not appear to influence the normal physiological processes in any way, and any significant results found were ones which could be easily accounted for by slightly different rates of increase for the particular groups and sexes involved. This would indicate that although MDMA could play a significant role in affecting weight gain and associated physiological processes, this is only relevant at certain points of exposure to the drug – such as early adolescence.

When the rats had reached full maturity, post-testing body weights showed further differences accounted for by MDMA. In adulthood, MDMA-treated animals weighed significantly more than controls. Overall, males weighed significantly more than females. Male rats treated with saline during late adolescence weighed less in adulthood than those that had been treated during early adolescence. Male rats treated with MDMA weighed more than controls only when treated
during late adolescence, whereas female rats treated with MDMA weighed more than controls only when treated during early adolescence. This would suggest that the weight of an animal even in adulthood was able to be accounted for by earlier exposure to MDMA, but that both the period of administration and the sex of the animal played a significant role. Female rats appear to be more susceptible to weight gain accounted for by MDMA in early adolescence, whereas male rats appear to be more susceptible to weight gain accounted for by MDMA in late adolescence.

6.1.3 Open-Field Test

Results from the open-field apparatus showed significant effects of MDMA on emotional reactivity on several measures. Ambulation was seen as being indicative of heightened levels of activity and exploration, and an increase in ambulation suggestive of a lowering of emotional reactivity. Treatment with MDMA resulted in decreased ambulation, indicating an increase in emotional reactivity, but only in rats treated during late adolescence.

Male rats were shown to have lower ambulation levels than females regardless of MDMA administration. This finding was consistent with prior research, and indicative of greater general emotional reactivity among males (Archer, 1975; Palenicek et al., 2005).

Data from the grooming measure of the open-field test was interpreted as the response being indicative of emotionality. The results show that MDMA (but not control) females displayed more grooming behaviour than males. This would indicate a higher emotionality in females than in males when treated earlier with MDMA.

Defaecation data from the open-field test was interpreted as more faecal boluses indicating higher emotional reactivity or anxiety. MDMA treatment resulted in an increase of faecal
boluses, and so an increase in emotionality accounted for by MDMA. Furthermore, male rats defecated more than females, suggesting higher emotional reactivity among males in general, which is consistent with prior research (Archer, 1975).

Corner square occupancy data of the open-field test was interpreted as higher occupancy being indicative of greater timidity. MDMA treatment resulted in a decrease of corner square occupancy, indicating a lowering of timidity following MDMA administration, and interpreted as a decrease in emotional reactivity accounted for by MDMA. This finding, however, is at odds with the other data. The lowering of corner square occupancy accounted for by MDMA could be explained by the corresponding increase of general activity resulting from MDMA intake, however this is not reflected in the ambulation data.

6.1.4 Light-Dark Choice

The light-dark box supplied significant results regarding effects of MDMA specifically among late adolescent rats. Movement between the lit and the dark sides of the light-dark box is indicative of greater exploratory behaviours, and thus is an inverse measure of emotionality. MDMA treatment resulted in a significant decrease in transitions between the two areas, but this was seen in rats treated during late adolescence only. This would suggest an increase of emotionality accounted for by MDMA in late, but not early adolescence.

Time spent in the lit area is also an inverse measure of emotional reactivity, where greater time spent in light indicates greater exploration and thus lower anxiety. The results show a significant increase from time spent in light in early adolescence to late adolescence for females only. This would suggest that the adolescent period is significant for female, but not male rats, with a decrease in emotional reactivity toward late adolescence.
6.1.5 Responsiveness to Brightness Change

The Y-maze measured general responsiveness to brightness change, and although the percent of entries of the novel arm as well as the percent of time spent in the novel arm proved to have no effects accounted for by MDMA, significant results were seen on other measures.

Total entries of both arms is a measure of exploration, inversely indicating anxiety or emotionality. MDMA treatment resulted in a significant decrease of entries of both arms, but for rats treated during late adolescence only. This suggests an increase of emotional reactivity accounted for by MDMA in late, but not early, adolescence. Furthermore, data from the control groups indicated that rats that had received treatment during late adolescence made significantly more entries than rats that had received treatment during early adolescence. This would suggest an effect on emotional reactivity accounted for by age at the time of treatment: rats that had received saline treatment in early adolescence were showing more anxiety than those treated in late adolescence.

Males were found to have made fewer entries than females, suggesting that males had greater emotionality, which is contrary to prior research (Hughes, 2001).

The total time spent in both arms is also a measure of exploration, which inversely indicates emotionality. MDMA treatment resulted in decreased time spent in both arms, indicating an increase of emotional reactivity following MDMA administration.

Males were shown to spend less time in both arms than females, suggesting that males were more emotionally reactive. These findings are contrary to prior research, where males were shown to spend more time in both arms (Hughes, 2001).
6.1.6 Summary

The results show that MDMA exposure in early and late adolescence resulted in a number of significant effects. The majority of these results showed changes in anxiety of the rats, measured largely through the open-field, responsiveness to brightness change, and the light-dark measures. Tasks involving spatial or working memory, and novelty preference, such as certain measures on the responsiveness to brightness and the object recognition tests, appeared unaffected.

MDMA administration was seen to increase emotionality in general, as seen through measures on both the open-field task and the responsiveness to brightness test. However the period of administration also appeared to play a significant role. Treatment with the drug throughout late, but not early adolescence, was shown to increase emotional reactivity. This finding was seen consistently across the open-field, responsiveness to brightness, and light-dark choice tests. Furthermore, rats that had received treatment during early adolescence showed greater emotional reactivity than rats that had been treated during late adolescence. As these results were seen following treatment with saline, that would suggest that the effect was due to the treatment process itself. This would indicate that the early adolescent period itself was vulnerable to long-term effects on emotional reactivity, with heightened anxiety among rats treated during early, but not late adolescence. However the late adolescent period was vulnerable to long-term effects on emotional reactivity accounted for by MDMA, with heightened anxiety among rats treated during late, but not early adolescence.

The sex of the animal also appeared to play a part. Results from the open-field test indicated that MDMA treatment among female rats showed a greater emotional response displayed through grooming behaviour in a stressful situation later in life, compared with male rats.
Furthermore, female rats that received any treatment during late adolescence showed a decrease in emotional reactivity, as indicated by the increase of the amount of time spent in the light side of the dark-light box by late adolescent females. This further supports the data showing a general decline in emotional reactivity, and thus heightened vulnerability among early adolescent rats compared to rats treated during late adolescence. Additionally, female rats in particular are implicated as being less susceptible than male rats to long-term effects following treatment.

Greater weight gain in adulthood was also influenced by MDMA in early adolescence for females only, and in late adolescence for males only. This is possibly due to different rates of physiological growth in males and in females, with one age group being particularly vulnerable to MDMA-induced loss of appetite and resulting weight loss, depending on the sex of the animal.

Testing on the open-field shortly after the final dose of administration showed that the drug was having an immediate effect on the rats when compared to the controls on several measures: rearing, grooming, and walking. Rats treated with MDMA during both early and late adolescence all displayed a significant decrease in rearing and grooming, and an increase in ambulatory behaviours. Rats treated during late adolescence only displayed MDMA-accounted for sex differences, with more grooming behaviours among female rats. The results support the effectiveness of the dose chosen for administration, and indicate that the rats displayed largely similar effects of the drug at the time of administration, regardless of their age, although there was a slight sex difference among the older rats.
6.2 METHODOLOGICAL LIMITATIONS

The dosing regimen of the subjects is one which would make it hard to draw comparisons with human studies, as it involved chronic administration of MDMA via daily intraperitoneal injections. This would be unlikely to occur among humans, where MDMA tablets are most commonly consumed orally, or nasally, and this occurs at more intermittent intervals. As the results indicated, the i.p injection alone was shown to create an anxiogenic effect, particularly among rats treated during early adolescence.

The open-field measure, although very widely used, draws on the concept of immobility indicating increased anxiety, whereas increased ambulation is indicative of decreased anxiety and reflects exploration of the novel environment. However similar behaviours may be described as escape behaviours, and indicative of increased anxiety (Archer, 1973). The open-field appears to have aversive properties which may counter the rat’s natural instinct to explore a novel environment (Crawley & Goodwin, 1980).

The process of cleaning the apparatus after each trial to remove any trace of faeces, urine, or residual smell of another rat, may mask anxiolytic behaviours. It is possible that a soiled apparatus reduces any neophobic response, and more clearly reflects influences of a wide open space, or light or dark areas on exploration activity (Bourin & Hascoët, 2003; Hascoët et al., 2001). However, as cleaning of apparatuses was consistent for all rats, any possible effect on anxiety was controlled across all trials for all animals. Still, results may have been slightly affected by the aversive smell of the cleaning process.

The use of behavioural observations only to draw conclusions is a limitation in terms of being able to draw more conclusive comparisons with prior research. Measuring serotonin levels
following the behavioural tests would have enabled a view of any neurochemical differences between the two developmental groups, as reduction of 5-HT is the most consistently identified long-term effect of chronic MDMA exposure (Piper, 2007).

6.3 METHODOLOGICAL STRENGTHS

The behavioural tests used have all been widely accepted as reliable measures of emotionality, and have been frequently used in similar research (Maldonado & Navarro, 2000; Meyer et al., 2008; Morley et al., 2001; Palenicek et al., 2005; Piper et al., 2005; Piper & Meyer, 2004).

The advantages of using rodents on long-term behavioural effects is the shorter life-span which makes it possible to observe and record drug-elicited effects in a controlled setting over a short period of time. Although the study used animal subjects, research has shown the possibility of applying animal research onto human responses to the same drug. This would suggest that the current study is able to be applied, to a degree, as a model of possible long-term effects of MDMA to be expected with human subjects, depending on the period of drug use.

Furthermore, this study was able to showcase the different long-term effects on anxiety accounted for by MDMA that were often sex- and age-dependent. This would suggest a similar effect may characterize other drugs similar to MDMA, and calls for further research on developing animals.

The use of female as well as male rats in the study showed significant sex differences present, and supports the need for further research using female subjects.
7.0 General Discussion

The results of this study provide sufficient data to be able to conclude with confidence that MDMA intake during adolescence (especially late adolescence) has significant effects which persist well into adulthood. To a greater or lesser extent, these effects appear to be largely dependent on the sex of the animal as well as the period of administration, further emphasizing the need for female as well as male participants to be used in research. Despite expectations, animals treated with the drug during early adolescence did not appear to be more affected than those treated during late adolescence, but rather the opposite, and would suggest greater plasticity among the early adolescent brain to the effects of the drug. However, the results showed that in general, animals that had received any treatment, saline or MDMA, during early adolescence had much greater long-term effects than those rats which had received treatment during late adolescence. This would indicate that the early adolescent period itself was vulnerable to long-term effects on emotional reactivity, with heightened anxiety among rats treated during early, but not late adolescence.

7.1 MDMA

MDMA has been a widely used drug for a number of years, and has spread to a much wider population, inclusive of young people. Its euphoric effects make it a popular drug most often taken at dance parties or clubs. The effects of MDMA on the brain have been widely studied, showing a considerable decline of dopamine, 5-HT, and 5-HIAA in various parts of the brain. Following the initial decline, cerebral 5-HT concentrations return to pre-treatment levels within 24 hours, but a week later decrease to less than 80% (Green et al., 2003). However although the
acute effects of MDMA are well documented, the long term effects have shown less consistent results. Chronic or repeated use of MDMA has been shown by some researchers to result in a range of cognitive and behavioural impairments, including deficits in memory, reasoning and anxiety up to two years later (Montoya et al., 2002).

The current study demonstrated no significant long-term effects on either spatial or working memory. However, there were significant impacts on anxiety or emotionality in adulthood following MDMA use mainly during late adolescence, with a marked increase of emotional reactivity accounted for by administration of MDMA.

However, as decreased levels of 5-HT following chronic MDMA exposure is the most consistently identified long-term effect, it demonstrates a limitation of the study (Piper, 2007). As serotonin levels were not measured, it becomes necessary to judge the extent of neural damage from behavioural observations alone. However as the cause of memory deficits has often been put down to the depletion of 5-HT levels, and memory does not appear to be affected, it could indicate that whatever serotonin levels had been affected during treatment had possibly sufficiently recovered (Montoya et al., 2002). This supports earlier findings which suggested that age is a factor in sensitivity to the long-term serotonin depletion following MDMA, where younger ages exhibit substantial and rapid neuroplasticity (Piper, 2007).

Furthermore, while males were found to be more emotionally reactive than females in general, MDMA administration resulted in an increase of emotionality in females compared to males. This corresponds with prior research, establishing that females seem to have a higher resistance to the consequences of MDMA (Piper, 2007).
7.2 ADOLESCENCE

Although MDMA use has been becoming more prominent amongst teenagers, the majority of studies have continued to focus on adult subjects. However, the effects of MDMA on a brain still undergoing neuronal maturation and completing its development may be significantly different from the effects of MDMA on a fully developed brain, particularly long-term.

Studies indicate that although brain development may be most pronounced throughout childhood, it does not end there. Rather, the brain continues to mature and develop throughout adolescence and into young adulthood. A range of cognitive changes occur, including a decrease in gray matter volume, cortical interconnectivity, and the prefrontal cortex (Rutter, 2007). These changes within the central nervous system result in the optimization of learning potential and the decline in rates of neuroplastic change (Chambers et al., 2003; Greydanus & Patel, 2005). These ongoing developmental changes result in a heightening of sensitivity to outside influences such as drugs, which may influence changes in neurotransmitter activity and the prevention of normal neurobiological development (Greydanus & Patel, 2005).

The current study indicated that when tested during adulthood, animals that had been treated during early adolescence showed greater emotional reactivity. This was regardless of MDMA administration, and appears to indicate that trauma earlier in life – such as daily i.p. injections – has long-term impacts on anxiety displayed in rats.

Comparisons between the early and late adolescent periods showed significant long-term effects of increased emotionality in adulthood when the drug was administered during late, but less so during early adolescence. However, in half of the significant outcomes, increased emotional reactivity was not dependent on the period of adolescence, and remained constant regardless on
the time of administration, and was accounted for through MDMA only. Nevertheless, there was enough significant data from the open-field showing decreased ambulation, from the light-dark box showing decreased entries of the lighter side, and from the responsiveness to brightness test showing decreased entries of both arms, witnessed only in rats which had been treated with MDMA during late adolescence, and all indicative of increased emotional reactivity and symptoms of anxiety.

Contrary to expectations, this then indicates that drug use during late adolescence has more profound long-term effects on emotionality than drug use during early adolescence. This may be explained by the ongoing development of the central nervous system, and the resulting neuroplasticity of the brain. The plasticity of the developing central nervous system may be more profound during an earlier age, and as such, a significant degree of serotonergic depletion may be possible to occur without any obvious impairments being visible until the extent of damage exceeds a certain threshold (Montoya et al., 2002; Piper, 2007). This suggests that the threshold is lower during late adolescence, and implies a greater resilience to MDMA or CNS plasticity during early adolescence.

This differentiates between data indicative of heightened emotional reactivity among rats which received any form of treatment in early adolescence, compared with rats which had been treated in late adolescence. The difference is likely to be due to different processes regarding trauma following i.p injections and handling early in life, and on effects of MDMA on the central nervous system. Early adolescent rats appear to be more vulnerable to any kind of treatment than late adolescent rats, whereas late adolescent rats appear to be more vulnerable to the effects of MDMA, both displaying long-term effects into adulthood.
No long-term effects were observed on measures of memory. These included the percentage of entries of the novel arm within the responsivity to change test, as a measure of short-term spatial memory, as well as the exploration of the novel object within the object recognition test, as a measure of working memory. This supported prior research, which demonstrated that the effects of MDMA on both learning and working memory appeared to be independent of the developmental stage of exposure, where adults were found to be more sensitive to the long-term depletions serotonin (Piper, 2007). This again is likely to be due to the developing brain being more able to compensate for serotonin deficiencies up to a certain point, due to the plasticity of the developing brain which becomes less pronounced in adulthood.

MDMA administration throughout late adolescence appeared significant for females, but not males. Female rats showed altered emotionality, specifically a decrease in emotionality. This suggests that the vulnerability of the developmental period is sex-specific, with females being more susceptible during late adolescence. More research is needed to further explore this area of sex-related developmental differences.

The current research looked at the long-term effects of prolonged MDMA use, involving i.p. administration of the drug once each day for 10 days. This in itself is not an accurate model of the average human use of MDMA, which tends to be oral, and administered sporadically, often only on the weekends. The use of intraperitoneal injection instead of oral administration in itself complicates pharmacokinetics by influencing metabolism rates, which are already vastly varied between human and animal subjects (Green et al., 2009). Despite these confounding factors, the study shows a clear difference between chronic MDMA intake during early and late adolescence, and female and male animals. It is possible to draw conclusions regarding late adolescence being more affected by MDMA in the long term regarding greater anxiety, possibly
accounted for by greater plasticity of the central nervous system during early adolescence, and the possibility of more effective recovery in the long-term. It also demonstrates a lack of long-term effects on working and spatial memory, despite the chronic dose of administration. These results are significant enough that although they may not be able to directly demonstrate the concurrent results in humans, the ethical limitations around designing a similar research using human subjects makes this research noteworthy. There are direct implications which suggest the possibility that similar results may be seen in human subjects.
8.0 Suggestions for Future Research

There has been a considerable amount of research on the effects of MDMA on both the adult human and rat brain. However, as already mentioned, there has been little research into the effects of MDMA on adolescents, a population of drug-users that is growing in size. The current research suggests that age of administration is a significant moderating variable that needs to be further explored. The use of both sexes highlighted clear differences present well into adulthood, and further support the significant gaps left behind by research using only male animals.

This study found significant long-term effects on emotionality but not on memory or cognition. However, as changes in these processes have been apparent in earlier research not concerned with adolescent development, it is possible that the measures of memory adopted in the present study were not sufficiently sensitive to detect any MDMA-related effects. More extensive research, including a neurochemical analysis of the brain, would be advantageous in exploring the long-term effects following MDMA use.

The period of drug administration used ranged for a period of ten days in either early or late adolescence. Although this was judged to be an appropriate, future research benefit from introducing a more intermittent model that more closely models human ingestion of the drug, while still retaining the emphasis on the differences between early vs late adolescent periods of administration (Meyer et al., 2008).

Although the focus in this instance was on the long-term effects of MDMA, it would be well worth exploring whether other drugs similar to MDMA would have similar effects. This would
enable determination of whether the current results are specific to MDMA, or whether they have a wider application to drugs which are similar in action.

9.0 Conclusions

Throughout the years the popularity of MDMA has been growing among younger populations of party-goers. The current research investigated the long-term effects of this recreational drug in rats of varying stages of development, measured through various behavioural tests. Exposure to MDMA showed an increase of emotional reactivity in general, however the age of exposure did appear to play a significant role. Treatment with the drug throughout late, but not early adolescence, increased emotional reactivity on a range of measures, suggesting vulnerability to the long-term effects of MDMA on emotionality among late adolescents only. This was considered to be due to age-specific sensitivity to long-term serotonin depletion and greater neuroplasticity and recovery rates among early adolescents, and a lower impairment threshold among late adolescents. Treatment of any sort however demonstrated increased emotional reactivity in early adolescent rats only, suggesting an overall vulnerability to the long-term effects on emotionality. Early adolescent rats appear to be more vulnerable to any kind of treatment than late adolescent rats, whereas late adolescent rats appear to be more vulnerable to the effects of MDMA, both displaying long-term effects into adulthood.

Male rats were found to be more emotionally reactive than female rats overall, but MDMA accounted for a significant increase of emotionality in females compared to males, suggesting a higher resistance to the consequences of the drug by females. Furthermore, treatment with MDMA during late adolescence showed decreased emotionality in females only, which implies
a sex-specific vulnerability of the developmental period, where females are more susceptible during late adolescence.

No long-term effects were observed on measures of memory, consistent with prior research that the effects of MDMA on both learning and working memory appear to be independent of the developmental stage of exposure. This is likely accounted for by the plasticity of the developing brain being more able to compensate for serotonin deficiencies in both early and late adolescence in terms of memory.
References


