UNIVERSITY OF CANTERBURY
Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

SEX DIFFERENCES IN THE BEHAVIOURAL RESPONSES TO OLANZAPINE IN RATS

A thesis submitted in partial fulfilment of the

Requirements for the Degree of

Master of Science in Psychology

By

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2017

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Acknowledgements

Firstly, I would like to thank Professor Rob Hughes, for your support, guidance and supervision throughout this project. I am grateful for the time and effort you have put in to assist me through the past year. Thank you for offering positing, constructive and encouraging feedback. I would also like to thank my associate supervisor, Professor John Dalrymple-Alford.

I would also like to thank the animal technicians, Neroli Harris, Silvana de Freitas Costa and Anya Armstrong, for your dedication and assistance throughout the year; I could not have completed this research without you.

Lastly, I would like to thank my family and friends for the continual support throughout the year.

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Abbreviations

5-HT	Serotonin
ANOVA	Analysis of variance
CYP	Cytochrome P450
DA	Dopamine
EPS	Extrapyramidal symptoms
NE	Norepinephrine
OLA	Olanzapine
PND	Postnatal day
SGA	Second generation antipsychotic
SSRI	Selective serotonin reuptake inhibitor

Abstract

There are significant differences in the pharmacokinetics, pharmacodynamics, brain structure, neurochemistry and sex hormones of males and females, yet females are precluded from preclinical studies, with conclusions drawn from male only results and applied to females. Thus, females are still treated with the same dose of many drugs, including antipsychotics, as males. To investigate whether any sex differences were evident in the responses exhibited by male and female rats after acute exposure to the commonly used atypical antipsychotic olanzapine, several behaviours were observed. These were anxiety, locomotor activity and spatial working memory, the effects of antipsychotics on which are controversial, and often unexplored in comparison to commonly researched extrapyramidal side effects. 80 PVG/c hooded rats were randomly assigned to one of four groups in which they were administered a low, medium or high dose of olanzapine, or vehicle for control animals at post-natal day (PND) 70 for 42 days. Immediately after 21 days of treatment at PND91, and after 42 days at PND112, subjects were tested in the zero maze, light-dark box, open field and the Y-maze. It can be concluded that there are marked differences in the behavioural responses to olanzapine in males and females, eliciting a clear anxiogenic effect for females in several measures, especially at higher doses with regard to males. There is strong evidence to suggest that olanzapine has an anxiogenic effect on anxiety-related behaviours, however anxiolytic effects are still apparent. Nevertheless, it is clear that it does have an effect on anxiety-related behaviour. Olanzapine also has an overall negative effect on locomotor activity over time, though this effect is dose dependent. With regard to spatial working memory, there are also considerably contradictory findings, with the suggestion of a negative effect of olanzapine on memory over time, however it appears that females may be more resilient to this effect than males. Overall, it can be concluded that the response of

males and females to olanzapine differs in many cases. These results may have implications for the use of the drug with humans as it has been proven that females do experience significantly more adverse effects of the drug with respect to males.

1 Introduction

1.1 Summary

There is substantial evidence to suggest that there are significant differences in the pharmacokinetics, pharmacodynamics, brain structure, neurochemistry and sex hormones of males and females. Despite this, females are treated with the same dose of antipsychotics as males. This is largely due to the fact that females are precluded from preclinical studies, with conclusions regarding the drug in question drawn from male only results applied to females, regardless of their differences. As a result, females have a higher risk for adverse effects and are likely to report an overall ineffectiveness of the drug. A better representation of females is essential to improve the safety and efficacy of various drugs, and therefore it is crucial that preclinical studies of drug effects include females, and that data is analysed by sex. Observing sex differences in behaviours such as anxiety, locomotor activity and spatial working memory are important as the impact of atypical antipsychotics for these behaviours is controversial. A thorough understanding of this impact will give researchers a clearer understanding of these effects, while controlling for sex-specific differences.

1.2 Differences between males and females

Sex is the classification of male or female based on genetic composition and reproductive organs and functions, while gender is a social construct, generally culturally determined, and is expressed in terms of masculinity, femininity, and the quality by which people identify themselves and how they believe others to behave (Soldin & Mattison, 2009; Soldin et al., 2011).

Sex differences in the responsiveness of both rats and mice to a number of behaviourally active drugs have been regularly reported for nearly 50 years. However, a large

number of researchers who use rodents use only males in their drug studies (Hughes, 2007). The Food and Drug Administration (FDA) have suggested that women are subject to more adverse drug effects than men, and the effects are more severe (Soldin & Mattison, 2009). Multiple studies suggest that women also have a 50-75% higher risk for adverse drug effects than men (Haack et al., 2009). Between January 1997 and December 2000, 10 drugs were withdrawn from the market; eight of the 10 were withdrawn due to evidence of greater risks to women (US General Accounting Office, 2001). With regard to antipsychotic medication, several studies have addressed the issue of potential sex differences on both behaviour and tolerability of the drugs, yet no studies have actively investigated this difference specifically on behaviour, with most examining physiological differences such as weight gain or metabolic parameters (e.g. Davey et al., 2012).

Various factors influence both circulating drug concentrations and concentrations at the site of action, and this determines the outcome of the drug (Hardman et al., 2001). Specifically, sex can influence how the body deals with a drug, as well as what the drug does to the body.

Men and women differ in response to drug treatment as a consequence of many factors, including sex-specific differences in pharmacokinetics and pharmacodynamics, and sex specific differences in parameters such as body weight, height, body surface area, total body water, and the amount of intracellular and extracellular water (Anderson, 2008; Soldin et al., 2011). On average, women have a lower drug clearance and/or a smaller volume of distribution, as they are generally smaller in size and have a different body composition compared to men. Therefore, the recommended dose of a drug may result in higher drug concentrations for women (Chen et al., 2000). Pharmacodynamic factors may also increase female sensitivity to a given drug. For example, concentrations of a given dose of a drug

would be similar in both men and women, however, the drug-related response is often greater in females compared to males (Coker, 2008).

In order for a drug to work, a minimum concentration at the site on which the drug exerts its action must be reached. This concentration must then be maintained within a given target range to allow for therapeutic efficacy (Soldin & Mattison, 2009). The quantity of the drug required to be effective is not far removed from the quantity that causes significant adverse effects. Therefore, maintaining this steady state is not as simple as administering one single dose of the medication. Many factors can affect the level of circulating drug concentrations, and it is becoming increasingly clear that men and women differ in their responses to drug treatment. For example, a man has an average body weight of 78 kilograms and 42 litres of total body water, while a woman has an average body weight of 68 kilograms and total body water of 29 litres (Soldin & Mattison, 2009). Drug effects on behaviour in women compared with men is still largely unknown. Therefore, it is essential that sex-related differences in drug pharmacokinetics and pharmacodynamics must be assessed. An understanding of these factors will result in safe and effective therapeutic drug concentrations.

1.2.1 Pharmacokinetics

1.2.1.1 Absorption and bioavailability

Drug absorption and bioavailability are influenced by drug- and route-specific factors, such as oral, intramuscular, intravenous or intraperitoneal administration (Soldin et al., 2011). Since oral drug administration is common in pharmacotherapy, it is important to consider the possibility that several physiological parameters can have a major impact on the rate of drug absorption. These include food interactions (e.g. grapefruit juice for certain drugs such as oral contraceptives), other impacts of oral contraceptives, lipid solubility and biliary secretion.

Gastric and hepatic enzymes and transport proteins that oral drugs interact with before reaching systematic circulation are critical parts of bioavailability (Giacomini et al., 2010; Kadono et al., 2010). These enzymes change across the course of development, forming the foundation for differences between males and females (Kennedy, 2008). These processes, both metabolic and transport, are crucial in determining the success or failure of drugs that are developed for oral administration. When successful, oral drugs are permeable, soluble and ineffectively metabolised by hepatic or intestinal enzymes. For example, the differences in the volume of distribution between males and females, alongside gastric alcohol dehydrogenase activity, can provide one reason as to why the bioavailability of alcohol is greater in females than it is in males (Parlesak, 2002). Transporter proteins also play an important role in the transportation of drugs into and out of cells, and are therefore involved in hepatobiliary and urinary excretion (Giacomini et al., 2010). Tissue distribution and elimination pathways, and consequently the efficacy and toxicity of drugs, can often be explained by transporter proteins.

Women generally have a smaller body surface area, and gastric fluid flow, gastric emptying, gut motility and cardiac output are all slower in women compared to men (Soldin & Mattison, 2009; Smith, 2010). As a consequence, women have slower passage into the small intestine, which is the main site of drug absorption; therefore women will have a slowed absorption rate compared to men. If male data are used when making generalisations to females, this may result in lower peak levels of drug than expected for women (Bennik et al., 1998; Lorena et al., 2004). Gastric acid levels are also generally lower in women than in men, and duration of gastric acid secretion and gastrointestinal blood flow are commonly longer for women (Beierle et al., 1999). Absorption is mainly affected by the speed of gastric emptying and the presence of other substances which change the gastric pH, for example, proton pump inhibitors. Depending on drug ionisation this may result in an altered absorption

of acids and alkali, by slowing down the absorption of acidic compounds or speeding up the absorption of alkaline compounds (Ghandi et al., 2004; Smith, 2010). Gut transit times, the time it takes to digest a compound, also differ significantly between men and women, mean transit times being shorter in men (44.8 hours) compared to women (91.7 hours) (Stephen et al., 1986).

1.2.1.2 Distribution

The distribution of a drug throughout the body is affected by multiple body composition factors. These differ between males and females. Sex differences in these parameters may account for differences in drug concentration at the target site and result in varying responses to the drug (Soldin & Mattison, 2009). Men have approximately 15% less body fat than women, and have more lean muscle mass (Smith, 2010). Total body water, extracellular and intracellular water, total blood volume, plasma volume and red blood cell volume are, on average, greater in men than women. This means that, for women, there is less volume available for a drug to be distributed (Smith, 2010; Soldin & Mattison, 2009). If the same dose of a water soluble drug is administered to a man and a woman, the volume of distribution will be increased in the man, thus decreasing drug concentration with respect to a woman (Soldin & Mattison, 2009).

Conversely, if both a man and a woman were exposed to the same dose of a lipid-soluble drug, a drug that readily enters body fat, the same parameters would mean an increase in the volume of distribution of the drug in females. This is due to increased body fat as a percentage of total body weight in women compared to men, which may increase the total amount of lipid-soluble, slowly metabolised drugs present in their body. Differences in body fat and organ blood flow for women have been implicated in a faster onset of action and

prolonged half-life, demonstrating a larger volume of distribution for lipid-soluble drugs compared to men (Houghton et al., 1992; Xue et al., 1997).

Antipsychotic medications are lipophilic. For women, this means that there will be a greater distribution of the antipsychotic drug throughout their body for the same dose of the drug compared to men. When fat-soluble compounds such as depot medications are administered over time, more depot will accumulate in women, and this may mean that in order to reduce the risk of side effects, women need a longer interval between depot doses than men (Smith, 2010).

1.2.1.3 Metabolism

The main enzymes involved in hepatic drug metabolism belong to the Cytochrome P450 (CYP) group. Sex-related differences in pharmacokinetics may emerge from discrepancies in the regulation of CYP enzyme expression and activity, and this may be related to endogenous hormonal influences (Soldin & Mattison, 2009). CYP1A2, 2C19, 2D6 and 3A4 are the most important hepatic enzymes for the metabolism of antipsychotic and antidepressant drugs (Prior & Baker, 2003). Differences between men and women have been reported for all of these enzymes, illustrating sex difference in hepatic drug metabolism (Thurmann & Hompesch, 1998; Beierle et al., 1999; Tamminga et al., 1999; Hagg et al., 2001; Meibohm et al., 2002; Parkinson et al., 2004). Higher activity in females of CYP3A4 and 2D6 have been shown in studies of sex-based differences in CYP activity, and lower activity has been shown for CYP1A2, 2C19, and 2E1 compared to males (Hagg et al., 2001; Meibohm et al., 2002; Parkinson et al., 2004). It has been established that, compared to those of males, women displayed a 20-30% increased clearance for drugs that were CYP3A substrates on average (Greenblatt & von Moltke, 2008). Higher plasma concentrations of

clozapine and olanzapine have been found in females, and this appears to be caused by lower CYP1A2 activity (Lane et al., 1990; Kelly et al., 1999; Gex-Fabry et al., 2005).

P-glycoprotein, a drug transporter protein, also appears to be implicated in drug metabolism. Men have more than twice as much of this protein compared to women, resulting in lower drug levels in the serum of women as more drug substrate is bound to this protein carrier (Meibohm et al., 2002; Fleeman et al., 2010).

Olanzapine and clozapine are metabolised by CYP1A enzymes, which has higher activity for males (Cooper at al., 1984). This means that there is a prolonged half-life and reduced elimination rate in females. Olanzapine also demonstrates significantly higher plasma levels for women due to higher activity of CYP3A4 and CYP2D6 in women (Soldin et al., 2011).

Due to increased body fat as a percentage of total body weight in women compared to men, women have larger lipid compartments, which leads to longer half-lives and therefore may result in the accumulation of lipophilic drugs, including antipsychotics. This suggests the need for longer dosing intervals, particularly during pregnancy (Pollock, 1997; Seeman, 2004). The menstrual cycle, pregnancy and menopause are three physiological states that exist for women that do not occur in men. These states may affect drug metabolism in women, and therefore medication may need to be altered during these phases (Smith, 2010).

1.2.1.4 Elimination

The kidney is the main organ for drug elimination. There are known sex differences in all three of the major renal functions, including glomerular filtration, tubular secretion, and tubular reabsorption. In men, renal clearance is generally higher than in women (Gaudry et al., 1993; Berg, 2006). Alterations in renal blood flow, glomerular filtration rate, hepatic blood flow, bile flow and pulmonary function have been found to change the excretion of a

drug during pregnancy. For example, maternal renal plasma flow increases from 500 to 700ml/min/1.73m^2, 1.44 times greater than a non-pregnant woman, and 1.1 times greater than a male (DeCherney et al., 2006). A 10% lower glomerular filtration rate in women (Gross et al., 1992) results in 40-50% higher plasma levels while treated with the SGA amisulpride (Muller et al., 2006). These factors may result in drug accumulation within the body, and therefore a significantly decreased renal clearance of antipsychotic drugs in women. For example, when matched for weight, a man will clear the drug faster and have lower plasma levels than a woman. It has been found that men clear olanzapine 38% faster than women, and therefore women are at an increased risk of adverse side effects because the drug stays in the body for longer (Smith, 2010). Consequently, women require less antipsychotic medication than men.

1.2.2 Pharmacodynamics

There appear to be significant sex differences with regard to cortisol. Women are more sensitive to cortisol suppression, and therefore they may also be more sensitive to the effects on basophils, a type of white blood cell that helps prevent blood from clotting too quickly and promote blood flow to tissues, and helper T lymphocytes, or cells that are involved in adaptive immune responses (Leblhuber et al., 1993; Tanaka et al., 1993; Vierhapper et al., 1998).

1.2.3 Sex-specific conditions affecting pharmacokinetics and pharmacodynamics

The influence of sex hormones and changes in sex hormone levels can impact the pharmacokinetics of a drug on physiological functions. Metabolic changes in women may be dependent on hormone levels such as increased levels or estrogen or progesterone, which can alter hepatic enzyme activity and therefore increase drug accumulation or decrease drug

elimination. These hormone levels fluctuate during the menstrual cycle, while using oral contraceptives, and during pregnancy or menopause (Soldin & Mattison, 2009). Throughout the menstrual cycle, sex hormone levels are associated with specific hepatic enzyme activation and the elimination rate of certain drugs. For example, during the follicular phase, caffeine elimination is higher, and is prolonged during mid-luteal phase (Buchanan et al., 2009).

Numerous physiological changes occur during pregnancy that have been shown to affect drug plasma concentrations. Volume of distribution can be affected by increased plasma volume, increases in extracellular and intracellular fluid and body water; cardiovascular plasma concentrations can be affected by plasma volume expansion, increase in cardiac output and regional blood flow changes, and decreases in binding proteins, prolongation of gastric evacuation time, liver CYP enzyme changes, and increased renal blood flow and glomerular filtration rate affect drug plasma concentrations (Mattison et al., 1991; Loebstein et al., 1997).

Gastric acid secretion and gastric motility alterations and a decreased rate of drug metabolism and renal elimination have been demonstrated for women. These factors are associated with changes in reproductive hormone levels during the menstrual cycle (Hutson et al., 1989; Kashuba & Nafziger, 1998). Due to these factors, drug levels in women may remain higher resulting in prolonged clinical and adverse effects. Therefore, it is worth considering that women may require a lower dose compared with men (Stahl, 1998).

1.2.4 Brain organisation and neurochemistry

There are several fundamental differences between males and females that are firmly established in biology. Although male and female brains are very similar, there are consistent differences that have critical implications for each sex. Brain sex differences uniquely affect

biochemical processes, may contribute to the susceptibility to specific diseases, and may influence specific behaviours (Ngun et al., 2011). In turn, these differences may impact how males and females tolerate specific drugs, and how drugs work within their brains.

Organisation of brain areas such as the preoptic area (Gorski et al., 1978), neocortex, hippocampus and the corpus callosum (Juraska, 1991) appears to differ between male and female rats, as does the organisation of the amygdala (Mizukami et al., 1983; Hines et al., 1992), and the activity of several neurotransmitters including serotonin (Carlsson & Carlsson, 1988; Zhang et al., 1999), and striatum and nucleus accumbens dopamine (Becker, 1999).

Sex differences are also demonstrated by two targets for selective serotonin reuptake inhibitors (SSRIs), the 5-HT_{1A} receptor and 5-HTT transporter. SSRIs are psychotropic drugs that are used to treat depressive, anxiety, and personality disorders. Throughout the cortical and subcortical brain regions, women have significantly higher 5-HT_{1A} receptor and lower 5-HTT binding potentials. They also display a positive correlation between 5-HT_{1A} receptor and 5-HTT binding potentials in the hippocampus (Jovanovic et al., 2008). These distinct binding potentials may therefore result in biological differences in the serotonin system, and consequently sex differences in psychiatric disorder prevalence, especially for depression and anxiety. Some atypical antipsychotics, such as aripiprazole, are partial 5-HT_{1A} agonists, which means that they bind to and activated the 5-HT_{1A} receptor, facilitating its action (Stark et al., 2007). 5-HT_{1A} receptor activation has been linked to increased dopamine release in the medial prefrontal cortex, striatum and hippocampus, areas in which increased levels of dopamine is useful in the treatment of schizophrenia (Li et al., 2004; Bantick et al., 2005).

Several neuroanatomical sex differences have been observed in the rat brain, including differences in synaptic patterns and neuronal density and the posterodorsal aspect of the medial amygdala is 85% larger in male rats (Hines et al., 1992). Similarly for humans, females have over 20% fewer dopaminergic neurons in the substantia nigra pars compacta

compared to men (Dewing et al., 2006). The substantia nigra is made up almost entirely of dopaminergic neurons and is involved in the control of motor activity (Groenewegen, 2003). In the amygdala, adult males have a larger medial nucleus compared to adult females (Mizukami et al., 1998).

With regard to neurochemistry, males and females demonstrate different patterns of transmitting, regulating, and processing biomolecules (Ngun et al., 2011). In an animal study, male rats have more norepinephrine (NE) in the amygdala and hypothalamus 25 days after birth, but the direction of this sex difference is reversed at day 300 (Siddiqui & Shah, 1997). Dopamine activity is upregulated only in male rats, and NE activity is increased only in females in response to chronic stress (Luine, 2002). In a study by Ingalhalikar et al. (2014), males had greater intra-hemispheric connectivity compared to females, who showed greater inter-hemispheric connectivity.

1.3 Importance of female models

Given this information illustrating the size of the difference between males and females in terms of drug pharmacokinetics and pharmacodynamics, there are still strikingly few attempts to resolve this through the inclusion of women in preclinical and clinical studies; many researchers continue to ignore females in their animal studies and study males exclusively (Hughes, 2007). This is alongside the fact that women are still underrepresented in preclinical and clinical trials, and there is an absence of comparative studies exploring sex differences in the efficacy and adverse effect profile of SGAs (Aichhorn et al., 2006).

Antipsychotic medication is developed and pre-clinically tested predominantly on young fit males, while young women of reproductive age have often been excluded from the drug development process. As a result, a large amount of the information available about antipsychotic effects and adverse effects is deduced from the effects of trials on young men.

It has been demonstrated that for some drugs there is more than a 40% difference in pharmacokinetics between women and men (Anderson, 2008); therefore it is probable that the biological differences between males and females do affect their response to antipsychotic medication.

This has resulted in a lack of data to evaluate possible sex differences in drug efficacy and adverse effects (Aichhorn et al., 2005). This means that caregivers and health professionals are often left without knowledge of the appropriate dose or use of the drug in women, and therefore must estimate based on the limited amount of knowledge available. The principles of clinical pharmacology and the pharmacokinetics of a drug should be understood, as they apply to a given drug when determining what to prescribe.

These sex differences in pharmacokinetic factors mean that for women, the effective dose of many psychotropic drugs is lower compared to that of men (Melkersson et al., 2001). In studies involving first-generation antipsychotics, it has been found that a dose must be one and a half to two times higher in men compared with women to achieve a similar clinical response (Arnold et al., 2004).

Considering that behavioural pharmacological research with laboratory animals aims to produce ideas about the relationships between drugs and behaviour that may have research inferences for understanding the pharmacotherapy of human disorders that define both sexes, using male rodents only could be limiting, given the information that males and females differ considerably in their pharmacokinetic and physiological characteristics (Hughes, 2007). For example, even though anxiety disorders are revealed to be more common amongst females than males (Blehar, 1995), most rodent studies investigating the effects of anxiolytic drugs have involved males only, even given that fear responses in animals are analogous to anxiety reactions in humans (Palanza, 2001). Therefore, it is likely that these studies which use male rodents only may not have the same degree of relevance to women as to men.

One reason why female rodents have been ignored is the widely held belief that their oestrous cycle may confound the effects of an experimental manipulation (Meziane et al., 2007). Hormonal changes during the cycle do indeed affect how females may react to a drug; for example, amphetamine increases the locomotor stimulant effect during the oestrous cycle in female rats, suggesting potentiation of striatal dopamine release by oestrogen or progesterone (Becker & Cha, 1989). Male hormones, however, can also interact with drug effects. For example, testosterone was found to increase ethanol-related aggression in male mice (DeBold & Miczek, 1985). Furthermore, in order to successfully generalise results of drug effects on human females, investigating the specific changes in behaviour produced by hormonal influences is of considerable importance to establish an accurate representation of these effects.

Given this information, it seems useful to include females in studies of drug effects, monitoring their oestrous cycle changes if the associated hormonal state is believed to be important. Male-only studies of drug effects may be irrelevant as the assumption that both males and females will demonstrate the exact same behaviour as that observed of males is not a reliable model of behaviour for females (Hughes, 2007). It appears that sex differences in animal models may be more relevant to the clinical setting than previously thought (Weston-Green et al., 2011).

The prevalence of psychiatric disorders also differs between males and females, and both men and women may present their symptoms differently. Specifically, schizophrenia research has showed that this disorder follows a different course in females compared to males, and this may have an impact on both the preferred treatment and the treatment outcome (Aichhorn et al., 2006).

It is important to look at more personalised medicine concepts instead of the 'one-drug-fits-all' approach, especially given that more than 70% of schizophrenia patients treated with SGAs discontinue their medication within 18 months of treatment because of the side effects accompanying drug treatment (Lieberman et al., 2005). Current evidence is derived from small studies that had not originally been designed to assess sex differences. Therefore, further studies are needed, especially prospective trials on sex-related differences in specific drug side effects to confirm exploratory findings (Haack et al., 2009). Without these, it will be difficult to make generalisations to both male and female human populations based solely on male preclinical studies.

The inclusion of women in preclinical (and clinical) trials and analysing data by sex instead of generalising male findings to females will provide more information on drug dose, pharmacokinetics and pharmacodynamics, and therefore advance the understanding of drug efficacy and safety in women (Aichhorn et al., 2006).

1.4 Olanzapine

Second generation (or 'atypical') antipsychotics (SGAs) are the mainstay treatment for schizophrenia and bipolar disorder (De Oliveira & Juruena, 2006). They are a varied class of drugs, including olanzapine, clozapine, risperidone, and quetiapine, which are grouped together based on their lower risk for extrapyramidal symptoms (EPS), which are typically associated with their first-generation counterparts (De Oliveira & Juruena, 2006). A common characteristic of SGAs is prominent serotonin 5-HT_{2A} antagonism combined with dopamine D_2 antagonism, as the positive symptoms of schizophrenia are believed to arise from hyperdopaminergic activity (Karl et al., 2006). SGAs also have a unique receptor-binding profile for histamine, muscarinic, adenosine and serotonin receptors, the consequence of which can result in a combination of both distinctive and overlapping side effect profiles (Hill

et al., 2010). SGAs therefore have superior therapeutic efficacy in treating not only the positive symptoms of schizophrenia, which was the main target of conventional antipsychotics, but also the negative and cognitive symptoms (Conley & Mahmoud, 2001).

Alongside risperidone, olanzapine is one of the two most commonly prescribed atypical antipsychotics in New Zealand (Wheeler, 2006; Gee & Croucher, 2011; Monasterio & McKean, 2011). It is the most commonly prescribed atypical antipsychotic for treating bipolar disorder, and is often prescribed off-label for treating refractory depression and borderline personality disorder (Monasterio & McKean, 2011). In a recent study by Dey et al. in 2016, olanzapine had the highest prescription rate of 31% of 451 patients in New Zealand when compared to other second generation antipsychotics and conventional antipsychotics, including risperidone, quetiapine and haloperidol. In the United States and the United Kingdom in 2006, both olanzapine and risperidone were prescribed equally often, and olanzapine accounted for 65% of atypical antipsychotic prescriptions in Australia (Wheeler, 2006).

Olanzapine has been available in New Zealand since 1997 and is a second-generation antipsychotic, antimanic and mood stabilising medication that demonstrates a broad pharmacological profile across a number of receptor systems (MedSafe, 2017). Olanzapine has demonstrated a range of receptor affinities for serotonin (5-HT), dopamine (DA), cholinergic muscarinic receptors, α_1 adrenergic and histamine receptors. Consistent with the receptor binding profile, it is a 5-HT, DA and cholinergic antagonist. Olanzapine selectively reduces the firing of mesolimbic dopaminergic neurons, while having little effect on the striatal pathways which are involved in motor function. As a result, olanzapine has a lower risk for EPS. This medication reduced a conditioned avoidance response (a test indicative of antipsychotic activity) at doses below those producing catalepsy (Medsafe, 2017). Olanzapine

has also been shown to increase responding in an anxiolytic test (MedSafe, 2017). It has been shown to significantly reduce negative as well as positive symptoms of schizophrenia.

Olanzapine reaches peak plasma concentrations after oral administration and absorption within 5 to 8 hours (MedSafe, 2017). It is metabolised by the liver by cytochromes P450-CYP1A2 and CYP2D6, where the main circulating metabolite is the 10-N-glucuronide which cannot pass the blood-brain barrier. The mean half-life of olanzapine is 33 hours, and mean plasma clearance is 26L/hr. Smoking status, age, and sex can vary the pharmacokinetics; for example, the mean half-life was prolonged in female subjects compared to males (36.7hr vs. 32.3hr) and clearance was reduced (18.9L/hr vs. 27.3L/hr) (MedSafe, 2017).

It is apparent that sex differences with regard to efficacy and adverse effects of SGAs have not been well studied to date, but it is clear that there are some disparities between males and females, and some physiological effects such as weight gain, hyperprolactinaemia and cardiac effects are particularly problematic for women (Aichhorn et al., 2007). Olanzapine appears to be associated with greater bodyweight gain than other SGAs, and serious side effects such as metabolic syndrome are more frequent in females, highlighting the need for further research.

Plasma levels are significantly higher in women because of increased CYP1A2 and CYP2D6 metabolism, and lower CYP1A2 enzyme activity. Therefore, dose-related plasma concentrations of olanzapine are significantly higher in women compared to men (Weiss et al., 2005). Olanzapine clearance is also higher in men (Callaghan et al., 1999). Strong drug-specific metabolic changes have been associated with olanzapine and quetiapine (Haack et al., 2009).

1.5 Behaviour

Several examples of behaviour that have been shown to produce sex differences in drug studies include motor activity, fear, and spatial learning (Hughes, 2007).

Numerous animal models are available for the study of various drugs, including SGAs. However, most behavioural studies focus only on EPS (E.g. Glenthoj et al., 1990; Drago et al., 1997) without looking at other behaviours such as anxiety, memory, fear, or general motor activity. Therefore, it would be difficult to cross correlate these behavioural findings (Karl et al., 2006). The effects of antipsychotic treatment on memory (Green et al., 1997; Wolff & Leander, 2003) and anxiety (Russell et al., 1987; Simon et al., 1993; Rodgers et al., 1994; Blin et al., 1996; Ishida-Tokuda et al., 1996; Singh et al., 1997; Nowakowska et al., 1999) are disputed in terms of whether there is a positive or negative effect, or no effect.

1.5.1 Anxiety

In a study by Karl et al. (2006), it was found that treatment with SGAs resulted in diminished motor activity and exploration, impaired working memory and increased anxiety levels. When comparing rats treated with SGAs to conventional antipsychotics and placebo, it was found those treated with SGAs showed more anxiety-like behaviours than controls, but fewer than rats treated with conventional antipsychotics (Hillert et al., 1992; Marder et al., 1997; Muller et al., 2010).

Many SGAs are often prescribed for treating anxiety-related disorders such as obsessive-compulsive disorder (OCD), post-traumatic stress disorder (PTSD) and panic disorder. Clozapine, olanzapine, quetiapine and risperidone have all been shown to alleviate the symptoms of these disorders, but there are several studies that have reported they can worsen the symptoms of the same disorders (Sun et al., 2010). Preclinical evidence of the anxiolytic property of SGAs is inconclusive, where various studies have found anxiolytic-

like, anxiogenic-like or no effect on anxiety-like measures (Karl et al., 2006; Sun et al., 2010). However, Mead et al. (2008) and Sun et al. (2010) found that rats treated with olanzapine, clozapine or risperidone showed anxiolytic-like behaviours which were not attributable to their antipsychotic effect or effects on motor functions or learning and memory processes, suggesting that olanzapine and risperidone may possess an anxiolytic-like property.

In spite of some negative evidence for the anxiolytic property of SGAs, there is a sufficiently large amount of evidence favouring olanzapine's dose-dependent anxiolytic action. Therefore, examining the effect of olanzapine on behaviour can further assist in understanding the anxiolytic-like property of this drug.

1.5.2 Locomotor activity

Olanzapine has been shown to significantly reduce locomotor activity, which may be described as a sedative effect of the drug (Evers et al., 2010; van der Zwaal et al., 2010; Muller et al., 2010). Consistent findings have been found in rats (Hillebrand et al., 2005; Stefanidis et al., 2009) and humans (Callaghan et al., 1997; Putzhammer et al., 2005; Roerig et al., 2005).

In a study by Davey et al. (2012), there was a reduction in locomotor activity, as observed in an open field test, in female rats treated with 2mg/kg olanzapine between 15 and 20 minutes and between 20 and 25 minutes. However, the female rats treated with 4mg/kg olanzapine only showed decreased locomotor activity between 15 and 20 minutes. Male rats treated with both 2 and 4mg/kg olanzapine exhibited reduced locomotor activity between five and 10 minutes, and male rats treated with 2mg/kg also showed reduced movement between 15 and 20 minutes and 20 and 25 minutes.

In a more recent study, rats treated with olanzapine showed reduced locomotor activity in terms of both total distance travelled and velocity throughout treatment periods of 8, 16, and 36 days (Qingsheng et al., 2014) as measured in an open field test.

Sedative side effects are common for antipsychotics (World Health Organisation [WHO], 1998), therefore a reduction in general motor activity is to be expected. However, the extent of this with relation to males and females is unclear.

1.5.3 Spatial working memory

There is a lack of information on the effects of SGAs on learning and memory in animal studies. One study by Skarsfeldt (1996) examined the effects of acute administration of several SGAs including olanzapine on learning of young adult rats in the water maze. It was found that quetiapine had no effect on spatial learning, clozapine impaired performance in the early trials but was ineffective during the later trials, and olanzapine and risperidone disrupted learning but not motor behaviour.

Another study by Rosengarten and Quartermain (2002) found that, when investigating the effects of chronic administration of SGAs on spatial learning and memory, risperidone and clozapine significantly disrupted learning processes, while olanzapine had no effect.

Terry et al. (2002) compared haloperidol with olanzapine for 45 and 90 days in a chronic study using the water maze task for effects on spatial learning and memory. After a 4-day drug washout period, previous exposure to neither haloperidol nor olanzapine for 45 days produced any significant effects on spatial learning in the water maze. However, after 90 days, both haloperidol and olanzapine resulted in a clear impairment of spatial learning. This result was replicated by Muller et al. (2010). It was also found that radial arm maze performance was impaired in rats treated acutely with olanzapine, clozapine and scopolamine (Ortega-Alvaro et al., 2005).

Working memory and attention are generally impaired in patients with schizophrenia, and since DA agonists may facilitate working memory, DA antagonists, such as typical antipsychotics, may impair working memory (Ortega-Alvaro et al., 2005). The performance of rodents in spatial memory tasks that are used to assess particular components of cognition involved in schizophrenia also appears to be disrupted by olanzapine (Muller et al., 2010). Because SGAs have fewer D_2 antagonistic properties compared to typical antipsychotics, they have been shown to improve working memory in schizophrenia.

1.6 Rationale, aims and hypotheses

As there is a relative lack of research investigating sex differences in the efficacy and safety of psychotropic drugs or studies including females in preclinical investigations of drug effects, the main aim of this study was to investigate whether any sex differences were evident in the behaviours exhibited by male and female rats after acute exposure to the commonly used atypical antipsychotic, olanzapine. Many studies analysing sex are purely reflective, and did not investigate distinctions between males and females. While there is evidence to suggest that males and females are remarkably different with regard to their pharmacokinetics, pharmacodynamics, brain structure and neurochemistry and sex hormones, in many instances, males and females are treated the same with regard to drug prescription for disease or disorder and drug dosage. In addition, females are shown to have higher risk for adverse effects than males (Soldin & Mattison, 2009; Haack et al., 2009).

Furthermore, it has been well documented that there are clear sex differences in the effects of many drugs, e.g. alcohol and antihistamines (Soldin et al., 2011). So it is important to consider that sex differences in the effects of other psychotropic drugs are not improbable.

Therefore, it was hypothesised that acute exposure to olanzapine would result in different behavioural responses between males and females, especially at higher doses. While

there is evidence of olanzapine-related effects on anxiety, locomotor activity and spatial working memory, none of the previously stated studies focused on sex differences in these behaviours or analysed their data by sex. In fact, most, if not all of these studies focused exclusively on one sex, with the majority using male rats only. Thus, while olanzapine-related differences have been found with regard to these behaviours, it is difficult to conclude that the results apply to both sexes, or to generalise findings to entire human populations. Anxiety was chosen as there is much controversy over whether atypical antipsychotics are anxiolytic, anxiogenic, or have no effect on anxiety. This study therefore aimed to clarify this issue, while determining if any sex difference in the results may account for some of this controversy. Although locomotor activity and spatial working memory have been shown to be impaired with SGAs, a third aim of the current study was to determine to what extent any impairments were dependent on the sex of the animal.

2 Methods

2.1 Subjects

The subjects were a total of 40 male and 40 female PVG/c hooded rats, which were bred in the Animal Facility of the Department of Psychology, University of Canterbury. All rats were weaned at 28 days old, and were assigned to cages in groups of three or four individuals of the same sex. This arrangement ensured sex differences in responsiveness to OLA could be analysed. All rats had free access to food and water, and were housed in a room with a 12-hour light/dark cycle, with lights on at 0800h. At the beginning of treatment, equal numbers of each sex were 70 days old, when the rats are young adults, as this age, on average, is the age humans will experience their first schizophrenic episode.

The care and experimental treatment of the rats complied with Parts 5 (Codes of Welfare) and 6 (Use of Animals in Research, Testing, and Teaching) of the New Zealand Animal Welfare Act, 1999, and had been approved by the Animal Ethics Committee of the University of Canterbury.

2.2 Drug and drug exposure

The drug studied was the second generation antipsychotic olanzapine, administered via drinking water. From records of the rats' bodyweights and daily water intake, it was calculated that, to achieve as near as possible the target doses of approximately 8, 12 and 16mg/kg/day (referred to as low, medium and high) olanzapine, the drinking solutions should contain 50mg/litre, 75mg/litre and 100mg/litre respectively for males, and 40mg/litre, 60mg/litre and 80mg/litre for females. This difference for the two sexes was to take account of the fact that, because female rats drink significantly more water/kg than males (due to their higher levels of circulating hormones associated with diuresis, McGivern et al., 1996), they need lower concentrations of olanzapine to achieve the same daily doses. The drug was dissolved in a 0.001M solution of acetic acid and diluted with tap water to administer the final dose. All animals were weighed every seven days and the drug was prepared and provided weekly, allowing drug intake over the previous week to be measured. Therefore, adjustments could be made to the solution depending on the amount of water consumed and the weight of the animals. In doing so, it was possible to ensure rats were receiving appropriate target doses.

Equal numbers of males and females were randomly assigned to one of four groups: low (8mg/kg/day olanzapine), medium (12mg/kg/day), or high (16mg/kg/day) olanzapine, or 0.1M acetic acid for control animals, to take account of any unanticipated effect of the vehicle. Final doses administered to experimental rats were approximate, and were as close as

possible to these target doses. Experimental rats were given free access to the drug or vehicle for 42 days. The target doses of olanzapine and the duration of treatment were based on its pharmacological and clinical profile, selected to closely represent the clinical use of olanzapine.

2.3 Behavioural testing apparatus

The zero maze was a circular apparatus with a circumference of 105cm that was raised 72cm from the ground. It was divided into four equal-sized quadrants – two closed, and two open. The two closed sections had a 25-cm-tall black metal wall on each side, whereas the two open sections had a low clear Perspex lip on each side that was 1cm tall. The floor in the closed sections was painted black, and in the open sections it was painted white.

The light-dark box comprised two 30 x 20 x 30 cm high compartments which were separated by a wooden partition containing a 10 x 10 cm opening that could be closed by means of a removable horizontal slide. Each compartment was covered by a hinged lid that was constructed from wood for the dark compartment and clear Perspex for the light compartment. The box sat on a 100 cm-high table beneath dim fluorescent lighting.

The Y maze was 10 cm wide and 14 cm high throughout, and consisted of a 30 cm stem and two 45 cm long arms with an angle of 120° between them. A transparent Perspex hinged lid covered the maze. Each arm contained a removable black or white aluminium insert, which occupied the width, height, and 40 cm of the length of the arms.

The open field was a wooden box that was 60 x 60 x 30cm tall. It comprised of a black painted wooden floor divided into 16 numbered squares (15 x 15cm) by means of a grid of white painted lines, four across and four down.

2.4 General procedure

Subjects were weaned at postnatal day (PND) 28. They were then housed in standard cages of same-sexed groups of three or four individuals with free access to drinking water. At PND70, treatment of olanzapine (low, medium or high) via drinking water for experimental subjects or 0.1M acetic acid via drinking water for control subjects began for a total of 42 days until PND112. All rats were weighed and their water intake measured every seven days. After 21 days of treatment at PND91 and 42 days of treatment at PND112, rats began their behavioural assessments on consecutive days. Each rat was tested in a zero maze, light-dark box, open field, and Y-maze, in order to assess any sex differences following olanzapine treatment that may manifest in the rats' behaviour, such as anxiety, locomotor activity, or working memory. Following each rat's trial, the apparatus was sprayed with a 4% solution of Powerquat Blue disinfectant, and wiped dry with paper towels. The order of testing was randomised for each animal as a counterbalancing measure, and continued until PND115.

In the zero maze, each rat was placed on a closed area of the maze and then it's entries into the open and closed areas were counted as well as, every three seconds (indicated by an auditory signal delivered through an ear-piece), whether it was occupying an open or closed area.

In the light-dark box, the rat was placed in the dark compartment and the time it took for it to first emerge into the light compartment it was recorded. This was followed by records of entries into and occupancy of (every three seconds) the light side of the apparatus.

In the open field, every three seconds it was noted which numbered square the rat was occupying, and if it was engaged in behaviour such as rearing or grooming. From the occupancy data, it was subsequently possible to count the number of times the rat was seen in one of the four centre or corner squares, and to also indirectly determine the distance

travelled (ambulation) by counting the number of times it was occupying a different square from that occupied during the preceding three second period (as per Hughes et al., 2014).

In the Y-maze, the number of entries into and time spent in each arm was recorded, as well as whether the first arm entered was novel or familiar. A three second timer was used so that behaviours could be recorded at regular intervals and after each individual trial, the apparatus were cleaned to stop olfactory smells interfering with behaviour.

The use of a momentary time-sampling procedure for recording behaviour in each type of apparatus is an accurate and reliable way of measuring frequencies and duration of responses (Powell et al., 1977; Detke et al., 1995).

2.4.1 Zero maze testing

Each rat was placed in a closed area of the zero maze. For five minutes, entries into and time observed in the open and closed areas were recorded. Entries were defined as a transition involving all four paws. The zero maze was developed to eliminate the ambiguous centre region of the elevated plus maze thereby allowing uninterrupted, continuous movement through the apparatus. Rodents have an innate tendency to avoid brightly lit areas and open spaces. The zero maze is a test of anxiety in rodents which has been pharmacologically validated with anxiolytic and anxiogenic drugs, and is based on this innate avoidance and on the spontaneous exploratory behaviour of rodents in response to mild stressors such as a novel environment, light, or both (Bourin et al., 2003; Acevedo et al., 2014; Kulesskaya & Voikar, 2014). Therefore, a natural conflict between the tendency of the animal to explore, and their fear/defensive behaviour to avoid the unfamiliar occurs when an animal is exposed to an unfamiliar environment or novel object (Bourin et al., 2003). When allowed to make a choice between two novel areas, closed areas are preferred (Braun et al., 2011). Therefore, percentage of time spent in the open areas reflects anxiety, where increases

in time spent in open areas are interpreted as decreased anxiety (Braun et al., 2011; Pahkla et al., 2000; Shepherd et al, 1994).

2.4.2 Light-dark box testing

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 was allowed free access to both compartments for five minutes. The latency of first entering the light side, the numbers of entries into and times observed in the light side were recorded. Entries were defined as a transition involving all four paws. As for the zero maze, higher anxiety is reflected in avoidance of the light side and preference for the dark.

2.4.3 Open field testing

Each rat was placed in the centre of an open field, a model that resembles the natural conflict in rats between the inclination to explore a new environment and to avoid bright, open areas. For five minutes, frequencies of behaviour the rat was engaged in, such as rearing, walking, grooming or remaining immobile, were recorded. Occupancy of each square involved all four paws. Occupancy of different squares was also noted, especially occupancy of corner and centre squares. This apparatus allows evaluation of locomotor activity, and it may also be used as an anxiety-related model. At the end of each test, the number of faecal boluses left in the open field were counted as another measure of anxiety.

2.4.4 Y-maze testing

Each rat was placed in the stem of the Y-maze, with one arm containing a white insert, and the other arm containing a black insert. After a five minute acquisition trial, in which the rat was allowed free access to both arms, the rat was removed and the black and

white inserts were replaced with two new clean black inserts. The rat was then immediately returned to the stem for a three minute retention trial. During this test, the number of entries into the changed and unchanged (novel/familiar) arms were recorded, as was the number of times observed in each arm and the stem. Entries were defined as a transition involving all four paws. The white or changed/novel arm was alternated for each trial as a counterbalancing measure. The Y-maze is used to assess spatial working memory in rodents by the pattern of arm entries the rodent makes. Continuous spontaneous alternation behaviour, a phenomenon based on rodents' tendencies to enter a less recently visited arm over the course of several consecutive entries, is achieved when a rodent enters a new or novel arm instead of returning to the arm visited previously on consecutive opportunities. In order for this to occur, the animal must remember which arm it previously entered, enabling it to alternate its choice on the subsequent trial. Ability to recognise the changed or novel arm is a measure of short-term spatial memory (Hughes & Maginnity, 2007). This phenomenon has been ascribed to several different mechanisms, including habituation to novelty, curiosity and a tendency to explore novel environments, and spatial working memory (Hughes, 2004; Heredia-Lopez et al., 2016; Bak et al., 2017). From the total entries and time spent in each arm, it is possible to calculate the time spent and percentage of entries into the novel arm, as opposed to the unchanged or familiar arm.

2.5 Statistical analysis

All responses were subjects to separate 4 (dose) x 2 (sex) x 2 (test) ANOVAs. Where appropriate, post-hoc comparisons between specific groups were made by Scheffe tests ($P<0.05$).

3 Results

3.1 Behavioural results

All outcomes for the effects of sex (male or female), treatment dose and test (test 1 and 2, repeated measure) are shown in Table 1. Actual doses of OLA were 6 (low), 11 (medium) and 15 (high) mg/kg/day.

Table 1.

Mean ± SEM Values of all Responses in the Zero Maze, Light-Dark Box, Open Field and Y-maze for Male and Female Rats in Each Treatment Group in the First Test and Second Tests at PND 91 and PND 112

Measure	Treatment					Sex			Test		
	Control	Low	Medium	High	F(3,156)	Male	Female	F(1,158)	Test 1	Test 2	F(1, 158)
	n=20	*n*=20	*n*=20	*n*=20		*n*=40	*n*=40		*n*=80	*n*=80	
Zero maze											
% Open Entries	33.01(4.29)	21.5(4.01)	21.32(3.97)	25.42(4.47)	1.726	21.58(2.97)	29.04(2.95)	3.211	25(2.95)	25.63(3.03)	0.023
% Open Observations[a]	7.68(1.55)	5.35(1.45)	4.23(1.11)	4(0.89)	1.767	4.61(0.87)	6.01(0.95)	1.224	4.38(0.78)	6.25(1.02)	2.196
Light-dark box											
% Light Entries	49.75(1.35)	51.74(0.35)	51.93(0.5)	50.72(0.28)	1.745	50.7(0.73)	51.37(0.23)	0.752	51.24(0.22)	50.84(0.73)	0.272
% Light Observations	30.98(2.13)	33.5(1.93)	42.23(2.08)	36.95(2.04)	5.689***	34.38(1.67)	37.45(1.32)	2.269	36.34(1.45)	35.19(1.58)	0.505
First Emergence Latency	18.56(3.8)	21.15(4.7)	11.23(1.69)	12.3(1.78)	2.166	18.7(2.67)	12.93(1.89)	3.875*	19.85(2.62)	11.68(1.88)	0.931
Open field											
Ambulation[b]	39(2.66)	37.33(2.01)	41.63(2.23)	37.5(2.17)	0.923	34.81(1.56)	42.91(1.54)	15.305***	41.49(1.54)	36.24(1.63)	6.429*
Rearing[a,b]	21.73(2.17)	18.83(1.6)	24.9(1.69)	18.83(1.64)	3.535*	20.56(1.34)	21.08(1.25)	0.092	22.18(1.33)	19.46(1.24)	2.565
Centre Occupancy	5.93(1.94)	3.93(0.43)	5.4(0.76)	4.3(0.43	0.719	4.74(0.39)	5.04(1.01)	0.074	3.93(0.29)	5.85(1.04)	3.061
Corner Occupancy[b]	63.6(2.64)	65.55(2.06)	60.6(1.77)	64.23(1.87)	1.022	66.03(1.4)	60.96(1.53)	5.975*	62.95(1.34)	64.04(1.64)	0.276
Grooming[a]	1.53(0.28)	3.65(0.86)	3.28(0.58)	2.55(0.41)	3.225*	2.14(0.33)	3.36(0.47)	5.529*	3.85(0.49)	1.65(0.27)	17.832***
Faecal Boluses	0.35(0.2)	1.18(0.42)	0.35(0.19)	0.58(0.24)	2.115	0.99(0.25)	0.24(0.12)	7.834**	0.79(0.23)	0.44(0.16)	1.706
Y-maze											
First Choice Novel[c]	1.05(0.06)	1.28(0.08)	1.25(0.07)	1.23(0.07)	1.413	1.14(0.04)	1.26(0.05)	1.197	1.2(0.05)	1.2(0.05)	1.051
% Novel Entries	63.15(2.75)	56.54(2.26)	57.05(2.44)	61.03(2.22)	1.71	59(1.94)	59.8(1.52)	0.07	59.4(2.09)	59.43(1.32)	0.005
% Novel Observations	58(4.04)	55.28(3.89)	56.44(3.42)	59.2(4.45)	0.22	56.57(2.99)	57.88(2.43)	0.14	56.57(3.13)	57.91(2.25)	0.143
Total Arm Entries	4.13(0.42)	4.38(0.38)	5.3(0.37)	4.45(0.36)	1.846	4.05(0.26)	5.08(0.26)	7.492**	4.25(0.29)	4.88(0.25)	2.762

*p<0.05, **p<0.01, ***p<0.001

[a]dose x sex interaction significant (see text), [b]sex x test interaction significant (see text), [c]dose x sex x test interaction significant (see text)

3.2 Zero maze behaviour

3.2.1 Open arm entries

As shown in Table 1, there were no significant effects of sex, drug dose or test for this measure. There were also no significant interactions between any of these independent variables.

3.2.2 Open arm observations

As shown in Table 1, there were no significant effects of sex, drug dose or test for this measure. However, there was a significant dose x sex interaction, $F(3, 144) = 2.743$, $p<0.05$, outlined in Figure 1. Female control rats were observed significantly more often in open arms than female rats in all other treatment groups, and control males and males in the medium and high treatment groups. Male rats in the low treatment group were observed significantly more often in the open arms than medium and high treated male rats, but not control rats. Control female rats were observed significantly more often in open arms than their male counterparts, while the reverse was true for rats in the low treatment group. There was no significant sex difference for medium or high treated rats.

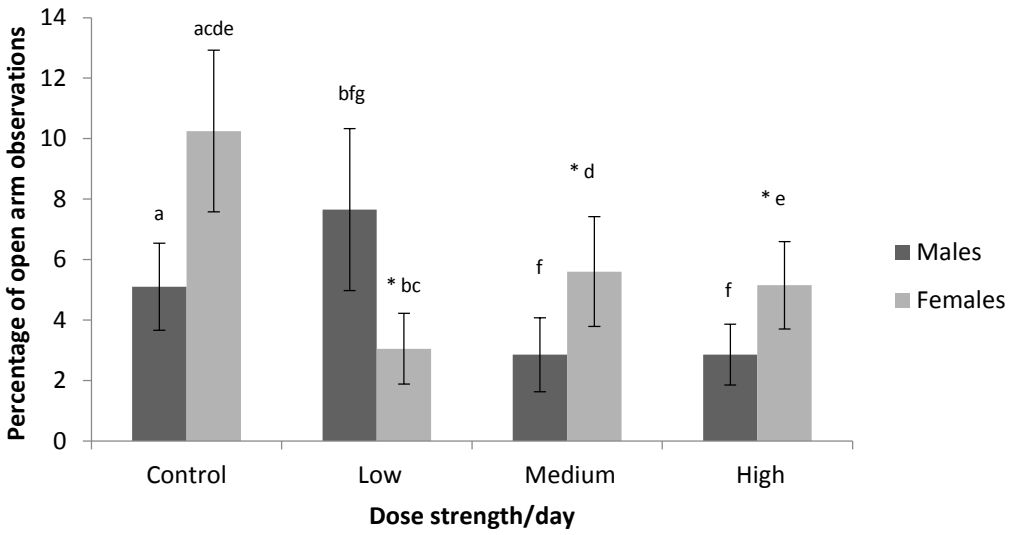

Figure 1. **Effect of sex and dose on open area observations in the zero maze. Error bars represent ±1 standard error of the mean.**

Note. abcdef difference between groups with superscripts in common significant (p<0.05). *difference between the drug group indicated and the control group significant (p<0.05) for that particular sex.

3.3 Light-dark box behaviour

3.3.1 First emergence latency

As shown in Table 1, there was a significant main effect of sex on first emergence latency in the light-dark box. Males took significantly longer to emerge into the light compartment than females. However, there were no significant main effects for dose or test, and there were no significant interactions between any of these independent variables.

3.3.2 Entries into light

As shown in Table 1, there were no significant effects of sex, drug dose or test for this measure. There were also no significant interactions between any of these independent variables.

3.3.3 Observations in light

As shown in Table 1, there was a significant main effect of dose on the percentage of observations in the light compartment. Rats in the medium dose treatment group were observed significantly more often in the light compartment than all other treatment groups (see Figure 2). Control animals were observed least often in light, significantly less than medium and high dose groups, but not significantly less than low dose treated rats. However, there were no significant main effects for sex or test, and there were no significant interactions between any of these independent variables.

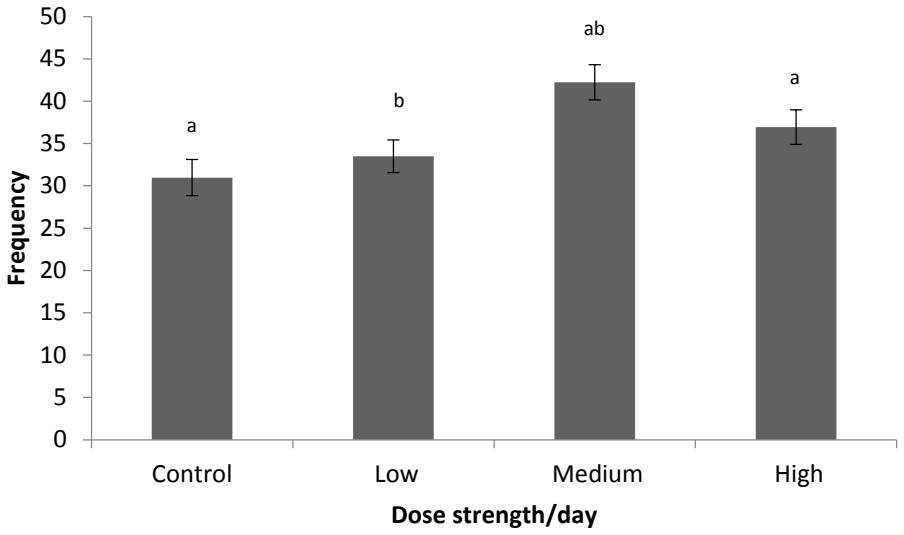

Figure 2. **Effect of dose on percentage of observations in the light compartment in the light-dark box. Error bars represent ±1 standard error of the mean.**

Note. ab difference between groups with superscripts in common significant (p<0.05). *difference between the drug group indicated and the control group significant (p<0.05).

3.4 Open field behaviour

3.4.1 Ambulation

As shown in Table 1, there was a significant main effect for both sex and test on levels of ambulation in the open field. Females emitted significantly higher levels of ambulation compared to males, and all subjects ambulated significantly more often in the first test than in

the second. However, this latter result is more appropriately considered in the light of a significant dose x test interaction, $F(3, 144) = 5.508$, $p<0.001$, outlined in Figure 3. In the first test, control animals showed significantly higher levels of ambulation than animals treated with any of the three doses of OLA. In the second test, control animals ambulated significantly less frequently than rats in the medium and high treatment groups. Control rats' activity decreased significantly between test 1 and 2; there was a nonsignificant decrease in low and high-dose treated rats' activity, and a nonsignificant increase in medium-dose treated rats' activity.

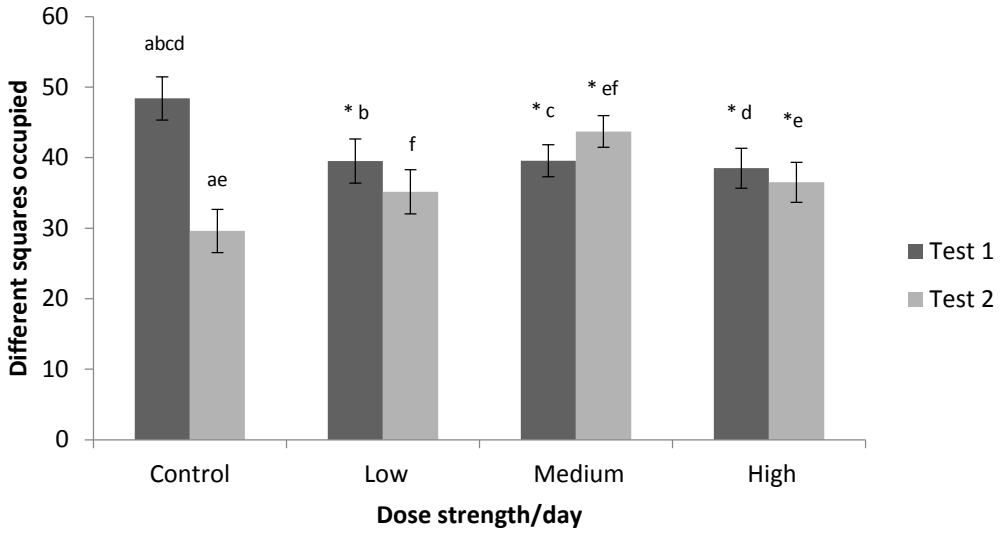

Figure 3. **Effect of dose and test on ambulation in the open field. Error bars represent ±1 standard error of the mean.**

Note. abcdef difference between groups with superscripts in common significant ($p<0.05$). *difference between the drug group indicated and the control group significant ($p<0.05$) for that particular test.

3.4.2 Rearing

As shown in Table 1, there was a significant main effect for dose on rearing in the open field. This may be better described in terms of a significant dose x test interaction $F(3, 144) = 4.077$, $p<0.01$ (see Figure 4). In the first test, control rats reared significantly more often than all drug treated rats. In the second test, rats in the medium-dose group reared significantly

more than low-dose, control, and high-dose treated rats, and rats in the high-dose treatment group reared significantly less than control rats. Rearing significantly decreased from test 1 to test 2 for both control rats and rats in the high-dose group, however, there was a nonsignificant increase in rearing for low and medium-dose treated rats. There was also a significant dose x sex interaction, $F(3, 144) = 3.822$, $p<0.01$. Females in the medium-dose group reared significantly more often than rats treated with low and high-dose OLA, as did control rats (see Figure 5). However, control males reared significantly less frequently than their female counterparts, and high-dose males reared significantly more often than their female counterparts.

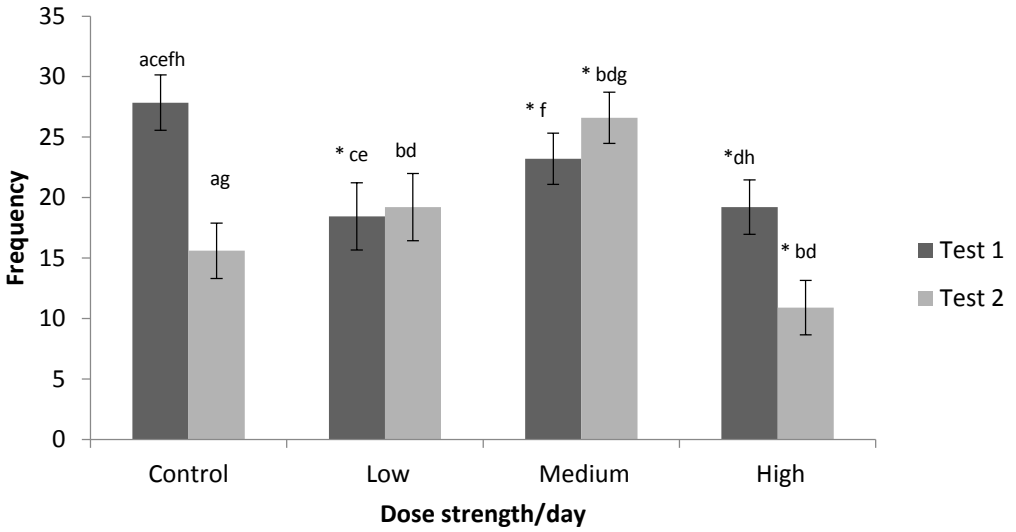

Figure 4. **Effect of dose and test on rearing in the open field. Error bars represent ±1 standard error of the mean.**

Note. abcdefgh difference between groups with superscripts in common significant (p<0.05). *difference between the drug group indicated and the control group significant (p<0.05) for that particular test.

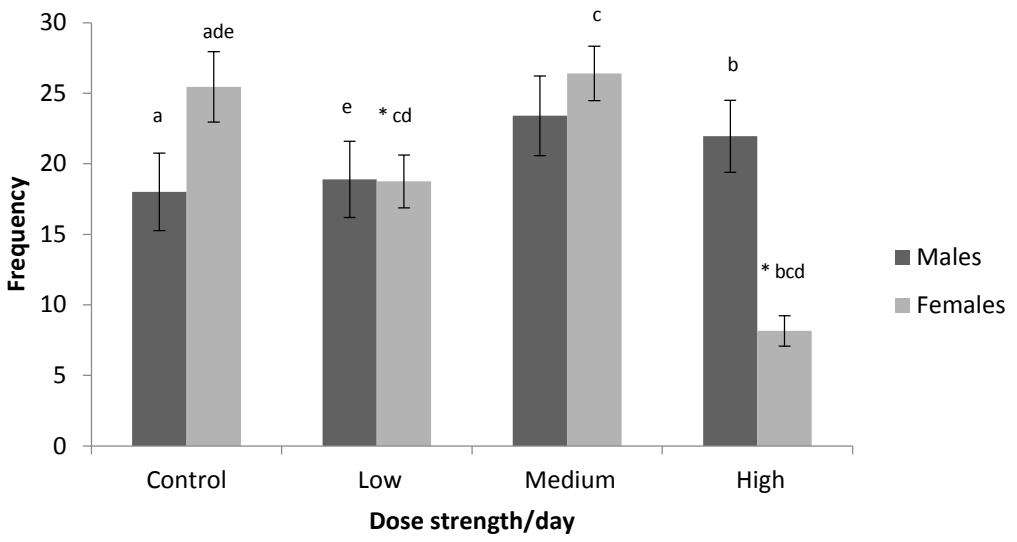

Figure 5. **Effect of dose and sex on rearing in the open field. Error bars represent ±1 standard error of the mean.**

Note. abcde difference between groups with superscripts in common significant (p<0.05). *difference between the drug group indicated and the control group significant (p<0.05) for that particular sex.

3.4.3 Centre occupancy

As shown in Table 1, there were no significant effects of sex, drug dose or test for this measure. There were also no significant interactions between any of these independent variables.

3.4.4 Corner occupancy

As shown in Table 1, there was a significant main effect of sex on corner occupancy in the open field. Males spent significantly more time in corner squares than females. There was also a significant dose x test interaction, $F(3, 144) = 2.65$, $p<0.05$. In the first test, control animals spent significantly less time in the corner squares than rats in the low and high-dose group. In the second test, control rats spent significantly more time in the corner squares compared to rats in the medium-dose. Occupancy of corner squares increased significantly between test 1 and 2 (see Figure 6).

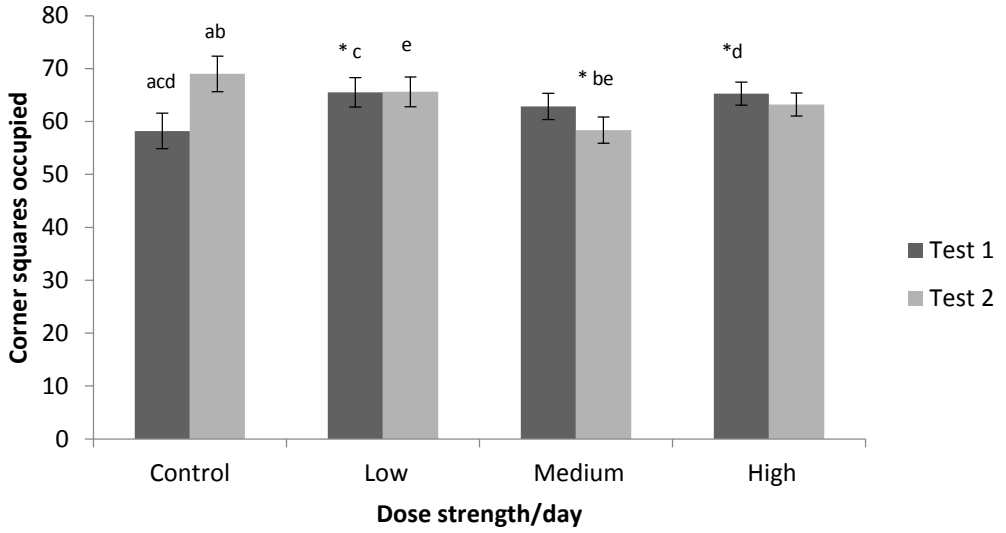

Figure 6. **Effect of test and dose on corner occupancy in the open field. Error bars represent ±1 standard error of the mean.**

Note. abcd difference between groups with superscripts in common significant (p<0.05). *difference between the drug group indicated and the control group significant (p<0.05) for that particular test.

3.4.5 Grooming

As shown in Table 1, there was a significant main effect of test on grooming. Grooming significantly decreased between test 1 and test 2. There were also significant main effects for sex and dose on grooming. These results may be better described in terms of a significant sex x dose interaction, $F(3, 144) = 4.597$, $p<0.01$. Control male rats groomed significantly less frequently than male rats in the medium and high-dose group. Low-dose treated females groomed significantly more frequently than control, medium and high-dose treated rats. Control females and low-dose treated females groomed significantly more often than their male counterparts (see Figure 7).

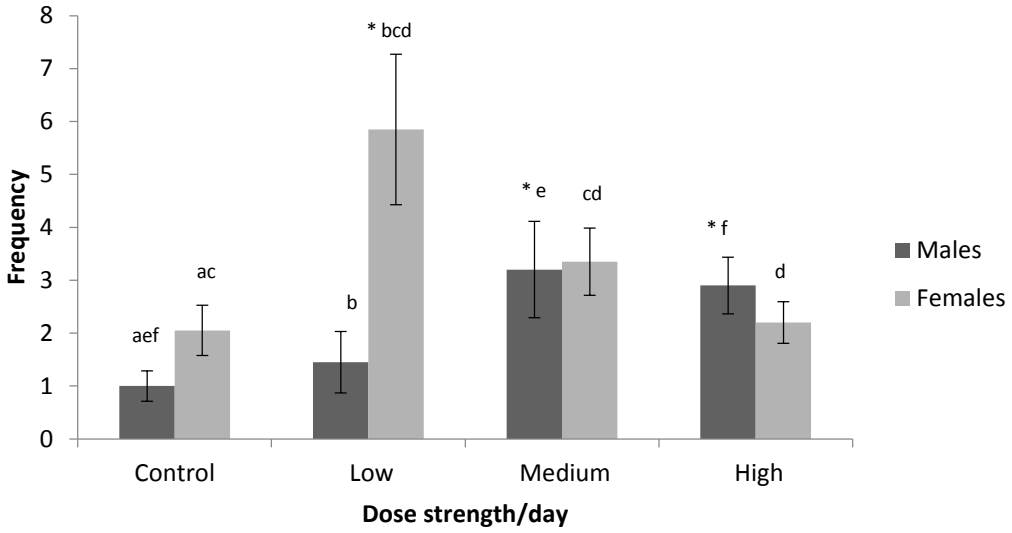

Figure 7. **Effect of sex and dose on grooming in the first test in the open field. Error bars represent ±1 standard error of the mean.**

Note. abcdef difference between groups with superscripts in common significant (p<0.05). *difference between the drug group indicated and the control group significant (p<0.05) for that particular sex.

3.4.6 Faecal boluses

As shown in Table 1, there was a significant main effect of sex on faecal boluses. Males defecated significantly more often than females. However, there were no significant effects of dose or test on this measure and there were no significant interactions between any of these independent variables.

3.5 Y-maze behaviour

3.5.1 First choice novel arm

As shown in Table 1, there were no significant effects of sex, drug dose or test for this measure. However, there was a significant sex x dose x test interaction, $F(3, 136) = 3.115$, $p<0.05$, (see Figures 8 and 9). In the first test, female rats in the low treatment group chose the novel arm first significantly more than male rats in the low treatment group. Female rats in the low treatment group also chose the novel arm first significantly more than females in the high treatment group, however males in the medium treatment group chose the novel arm

first significantly more often than males in the low treatment group. In the second test, control males chose to enter the novel arm first significantly less often than males in the low and high treatment groups, but not males in the medium treatment group. There were no significant results for females in this test. As seen in Figure 10, there was a significant decrease in frequency of choosing the novel arm first between the first and second tests for control males and males treated with a medium dose of OLA, and a significant increase for males treated with a low dose and females treated with a high dose of OLA.

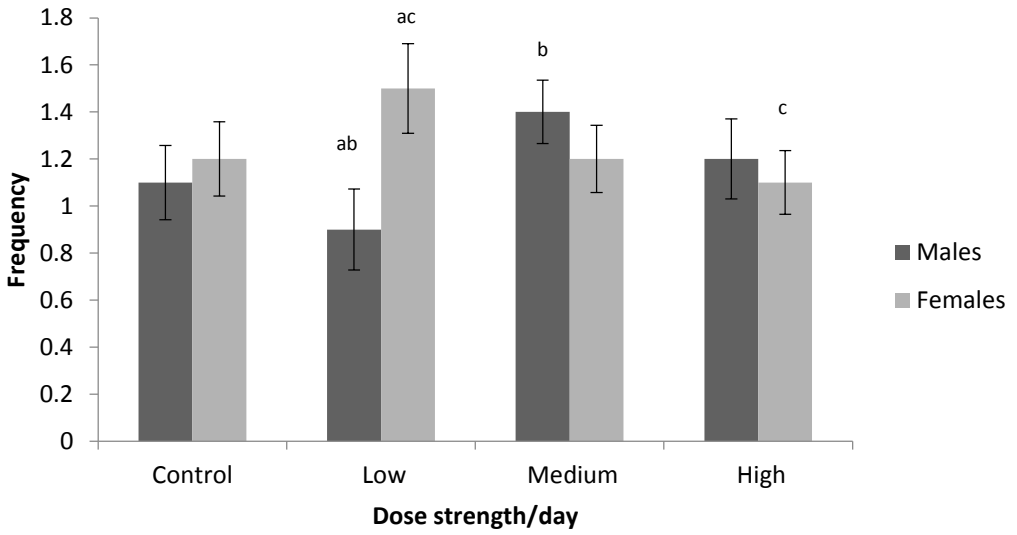

Figure 8. **Effect of sex and dose on first choice novel arm in the Y-maze in Test 1. Error bars represent ±1 standard error of the mean.**

Note. abc difference between groups with superscripts in common significant ($p<0.05$). *difference between the drug group indicated and the control group significant ($p<0.05$) for that particular sex.

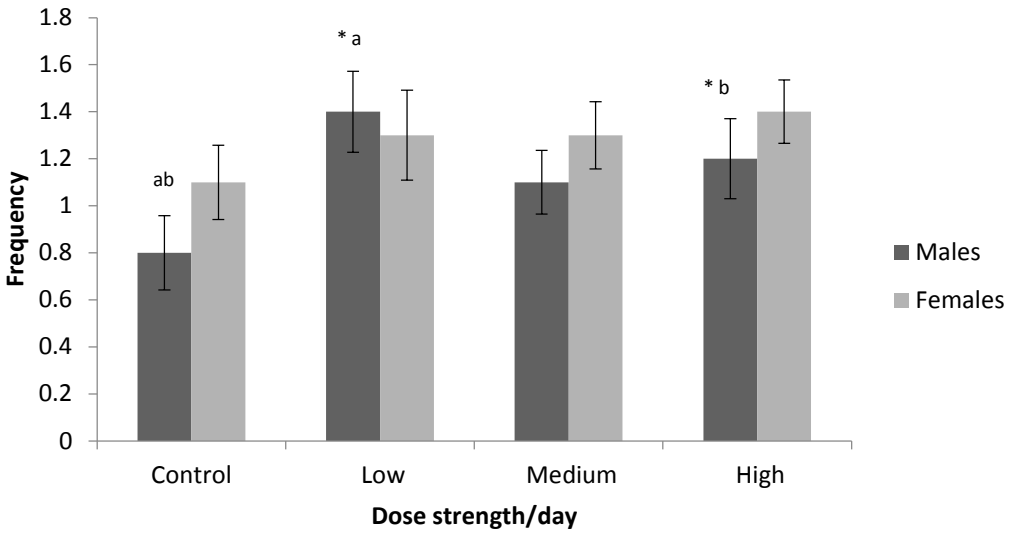

Figure 9. **Effect of sex and dose and test on first choice novel arm in the Y-maze in Test 2. Error bars represent ±1 standard error of the mean.**

Note. ab difference between groups with superscripts in common significant (p<0.05). *difference between the drug group indicated and the control group significant (p<0.05) for that particular sex.

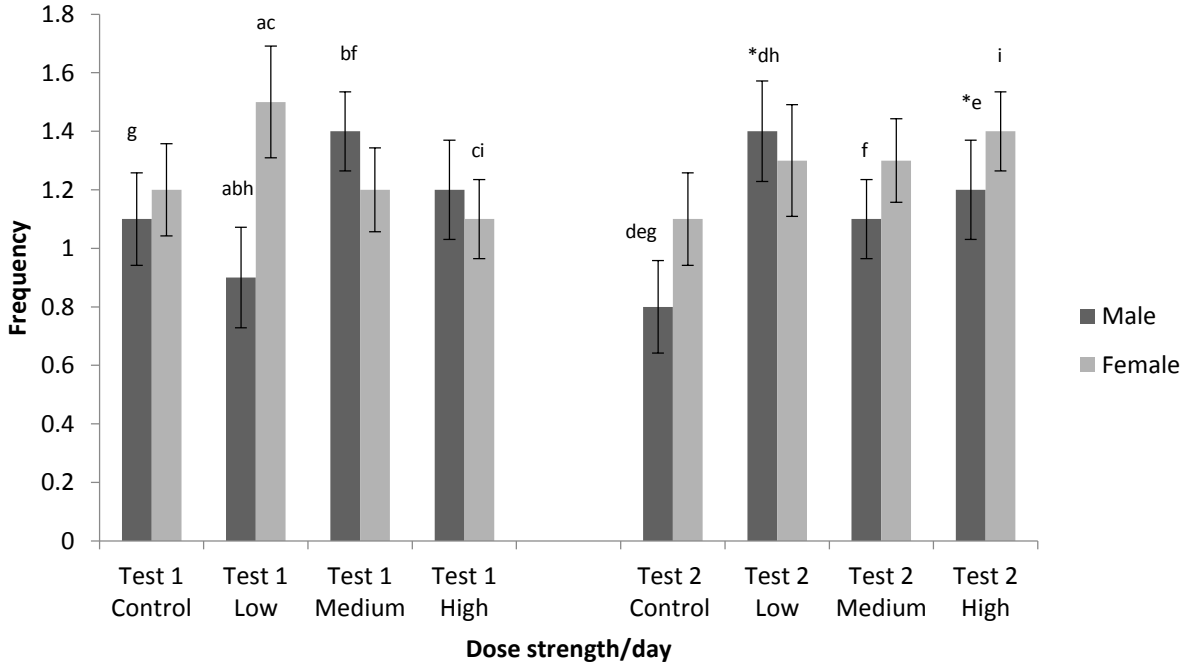

Figure 10**. Effect of sex and dose and test on first choice novel arm in the Y-maze for tests 1 and 2. Error bars represent ±1 standard error of the mean.**

Note. abcdefghi difference between groups with superscripts in common significant (p<0.05). *difference between the drug group indicated and the control group significant (p<0.05) for that particular sex for each test.

3.5.2 Novel entries

As shown in Table 1, there were no significant effects of sex, drug dose or test for this measure. There were also no significant interactions between any of these independent variables.

3.5.3 Novel observations

As shown in Table 1, there were no significant effects of sex, drug dose or test for this measure. There were also no significant interactions between any of these independent variables.

3.5.4 Total arm entries

As shown in Table 1, there was a significant main effect of sex on total arm entries in the Y maze. Female rats made significantly more total arm entries than male rats. However, there were no significant effects for dose or test, and no significant interactions between any of these independent variables.

4 Discussion

4.1 Overview

The current study sought to examine whether there were (and to what extent there were) any sex differences in the behaviours exhibited by male and female rats after acute exposure to the commonly used atypical antipsychotic olanzapine. This study also aimed to clarify the issue surrounding whether atypical antipsychotics are anxiolytic, anxiogenic or have no effect on anxiety, and to determine the extent to which SGA-related impairments in locomotor activity and spatial working memory differ dependent on the sex of the animal.

The dose (OLA, described below) and testing age (PND91 or PND112) were also examined as independent variables. At PND70, subjects were administered either approximately 0, 8, 12 or 16mg/kg olanzapine. Actual approximate doses were 0, 6, 11 and 15mg/kg OLA, referred to as control, low, medium or high dose OLA. Their behaviour was then tested in the zero maze light-dark box, Y-maze and the open field at PND91 and PND112 to assess differences in anxiety, working memory and general motor activity. It was hypothesised that acute exposure to olanzapine will result in different behavioural responses between males and females, especially at higher doses.

4.2 Olanzapine effects

4.2.1 Zero Maze

As illustrated in Figure 1, male rats in the low dose OLA group were observed significantly more often in the open areas of the zero maze than both medium and high dose treated males. This indicates that, for males, a lower dose of OLA is associated with lower levels of anxiety when compared to higher doses, as male rats in this group spent more time in the aversive open area. However, a clear conclusion of the nature of OLA cannot be drawn for males as all three treatment groups were not significantly different from control males. Nevertheless, males in the low dose treatment group were observed significantly more often in the open areas than females in the same group, suggesting that for this particular dose, males were less anxious than females. There were no significant differences in anxiety-related behaviour between males and females for any other doses. In contrast, control females spent significantly more time in the open areas of the maze than both control males and all other female treated rats. This suggests that at baseline, females are less anxious than males, and that OLA treatment has an anxiogenic effect on females, as its administration reduced the percentage of open area observations for this measure.

These results suggest an overall anxiogenic effect of OLA for females, as lower doses are associated with increased frequency of open area observations, however there is no clear effect for males. In general, these findings suggest an anxiogenic effect of olanzapine. Karl et al. (2006) found that antipsychotic medications such as haloperidol and risperidone increased anxiety levels in rats, as shown by a decrease in time spent in the aversive areas of the open field and the elevated plus maze and mixed findings have been found for the anxiety effect of olanzapine on obsessive-compulsive disorder (OCD), posttraumatic stress disorder (PTSD) and panic disorder. Evidence suggests that while olanzapine, clozapine, quetiapine and risperidone have been seen to improve symptoms, it has also been shown that the same SGAs also worsened symptoms in other cases (Moore et al., 1994; Brooke et al., 2005; Rogoz & Skuza, 2011). Olanzapine may exhibit a therapeutic efficacy in some forms of anxiety such as panic disorder, but there is also evidence to suggest that it is not effective in alleviating anxiety-like symptoms in generalised anxiety (Locchi et al., 2008). Overall, olanzapine presented as anxiogenic for females, however there was no clear response for males. This difference in behavioural response between males and females in this measure highlights the distinction between the two sexes and the need for further investigations into the extent that males and females differ in their drug response.

4.2.2 Light-dark Box

Rats in the medium dose treatment group were observed significantly more often in the light compartment than all other treatment groups (see Figure 2). Control animals were observed least often in light, significantly less than medium and high dose groups, but not significantly less than low dose treated rats. This suggests that rats treated with a medium dose of OLA were less anxious compared to rats from all other treatment groups, and control rats

and rats in the low dose group were the most anxious. This result indicates that OLA has an anxiolytic effect, as frequency of light observations, which are associated with decreased anxiety, increases with an increase in dose of OLA. Similarly, control and low dose animals were observed significantly less frequently in the light compartment.

This finding is consistent several studies reporting the anxiolytic effects of olanzapine (Mead et al., 2008; Sun et al., 2010). Frye and Seliga (2003) found that both 5 and 10mg/kg olanzapine has anxiolytic effects as it reduces fear and enhances social interactions in rats in relation to control rats due in part to its effects on serotonin and dopamine D_1 and D_2 antagonism. In the Geller-Seifter conflict task, olanzapine has also been found to increase licking of an electrified water bottle and inhibit the avoidance response of a conditioned avoidance task in rats, suggesting that it may have anxiolytic activity (Moore et al., 1992) and reduced the acquisition of conditioned freezing in male rats (Inoue et al., 1996). The anxiolytic effects of olanzapine may be due to its antagonistic effects on serotonin 5-HT_{2A} and dopaminergic D_1 and D_2 receptors. It has also been found that SGAs such as olanzapine activate brain areas involved in anxiety such as the medial prefrontal cortex and the locus coeruleus which in turn activated the hypothalamic-pituitary-adrenal (HPA) axis. The medial prefrontal cortex is also involved in working memory, learning, and emotional behaviour (Locchi et al., 2008).

4.2.3 Open Field

4.2.3.1 Anxiety effects

Rearing

In terms of rearing, mixed findings were present. As illustrated in Figure 4, in the first test, control animals reared significantly more often than all drug-treated animals, however

there was no significant difference between any of the three doses. This suggests that while the different doses did not have a significant effect, olanzapine in general did appear to reduce rearing. An increase in rearing is indicative of decreased anxiousness or increased exploratory behaviour and as OLA significantly reduced rearing for drug treated animals compared to controls, OLA appeared to have an anxiogenic effect in the first test. In the second test, control animals' rearing significantly decreased compared to the first test. Although there were no significant increases in drug treated animals' rearing, this increase in control animals' anxiety suggests that over time or with age, OLA does have protective effect on anxiety for low and medium dose groups only. There is also a significant decrease in high dose rats rearing which also indicates an increase in anxiety over time. This may suggest that low to medium doses of OLA are protective against increasing anxiety, however a high dose does not have the same effect. There may be a specific dose range that is anxiolytic. In general, this result suggests an overall anxiogenic effect of OLA in the first test, however for low and medium doses of OLA, there appears to be a protective (possibly anxiolytic) effect against an increase in anxiety over time or with age. This increased anxiety can be seen as a significant decrease in rearing for both control animals and animals treated with a high dose in the second test, with respect to the first.

There was also a significant sex x dose interaction effect, as illustrated in Figure 5. Female control animals were observed rearing significantly more frequently than rats treated with a low or high dose of OLA, but not a medium dose. This suggests that control animals were significantly less anxious than rats treated with a low or high dose, on which there appeared to be an anxiogenic effect of OLA, but at a similar frequency to rats treated with a medium dose of OLA, on which there was no significant effect. This result shows that there is no apparent trend in treatment and it is therefore not clear whether olanzapine has a direct

effect on anxiety for this measure, as both control animals and animals in the medium-dose group appeared to be less anxious than low and high dose groups. There were no significant differences in rearing behaviour for the different treatment groups for males. However, it was found that control females reared significantly more than control males and low-dose males. This suggests that at baseline or with a low dose of OLA, females appear to be less anxious than males as they exhibit higher levels of rearing. In contrast, males in the high-dose group reared significantly more often than their female counterparts, suggesting with a high dose of OLA, females appear to be more anxious than males. This is an interesting finding as it suggests that OLA has an anxiogenic effect on females at higher doses with respect to control. This may be an example of females needing a lower dose than males to avoid experiencing adverse effects such as increased anxiety. This also illustrates the sex differences in response to OLA, as there is a large disparity between male and female anxiety responses for this treatment group. This dose appears to have an aversive effect on females compared to males in the same group, and control females.

Corner occupancy

As shown in Figure 6, in the first test, low and high-dose animals were observed in the corner squares significantly more frequently than control animals, but not from each other or from medium dose animals. This suggests a general anxiogenic effect of OLA as it increased occupation of corner squares and therefore anxiety of two of the three OLA treatment groups. However, specific doses of OLA did not have a distinct effect on anxiety-like behaviour. Occupancy of the corner squares is considered an anxiety-related behaviour as it allows the rat to avoid aversive bright, open spaces. Increased time spent in these areas is indicative of an anxious rat. In the second test, control animals and low dose animals were observed significantly more frequently in the corners of the open field compared to medium

dose animals. There was not a significant reduction in corner occupancy for either the low or medium dose groups, however there was a significant increase in corner occupancy for control animals. This suggests that over time, controls became more anxious, and this increase can account for its difference with the medium dose group. Overall, there is no significant distinct change between any of the OLA treatment groups, suggesting a protective (possibly anxiolytic) effect from an increase in anxiety-related behaviour as seen in rearing. However, this is not a strong effect as there was no significant result for rats in the high dose group. Overall, in the first test, OLA appears to elicit an anxiogenic effect for low and high doses only, increasing the frequency of observations in the corner squares. However, in the second test, control animals' anxiety-related behaviour increases with respect to the medium dose OLA group, suggesting a possible protective effect of olanzapine. There were no significant increases in anxiety-related behaviour for the drug treatment groups. For control animals, age or repeated exposure to this test appears to have a negative effect on the animals.

In general for rearing and corner occupancy in the open field, these results suggest that olanzapine generally appears to have an anxiogenic effect in the first test and this effect becomes anxiolytic over time or with age in the second test. It is possible that this is protective effect, although there are very mixed findings. This may suggest that while initially anxiogenic, olanzapine stops an increase in anxiety-related behaviour. However, there were no significant decreases in anxiety related behaviour so it cannot be concluded that it reduces anxiety. This could be due to several factors, including the length of time since first olanzapine administration in the first and second tests, i.e. first administration was three weeks prior to the final administration, and pharmacokinetics may impact the behaviour elicited in response to OLA at various stages in this three-week administration, age, or greater familiarity of the apparatus. These results consistently suggest that olanzapine elicits an

anxiolytic effect over time or with age. It has been suggested that repeated treatment with olanzapine initially increases and then decreases the total density of $GABA_A$ receptors in the prefrontal cortex and thus suggesting an adaptive change over longer treatment periods (Locchi et al., 2008). Similarly, it is noted that olanzapine works by rebalancing dopamine and serotonin to alleviate the positive, negative and cognitive symptoms of schizophrenia and improve mood, thinking and behaviour, however the full benefit may not be seen for six to 12 weeks after first administration (Eli Lilly & Co., 2006; Li et al., 2007). This is not uncommon, as selective serotonin reuptake inhibitors (SSRIs), which are used to treat depression and anxiety disorders, often increase anxiety levels before the anxiolytic effect becomes evident (Mitchell, 2006; Harmer et al., 2009). Therefore, it may be that initial or early exposure to olanzapine as an anxiolytic increases anxiety before decreasing it. The different ages at which subjects were tested could also provide an explanation as to why olanzapine only appears to be anxiolytic in the second test. Although there is minimal evidence investigating the effects of age on olanzapine-induced anxiety, it is reasonable, based on the currently study, to suggest that an increase in age may be related to increased anxiolytic properties. However, this may also be due to the time at which the first dose of olanzapine was administered and may be purely coincidental. Further research would be beneficial here. Thirdly, there is the possibility of practice or learning effects and therefore the observed anxiolytic effect may be due to perhaps a decrease in curiosity or reduced anxiety upon re-exposure to the test. The previous experience rats had gained in this measure may in turn decrease their associated anxiety, as seen in several other studies (Rodgers & Shepherd, 1993; Aitchison & Hughes, 2006; Schneider et al., 2011).

Grooming

A dose x sex interaction effect on grooming in the open field showed that control male rats and low dose males groomed significantly less frequently than male rats in the medium and high-dose groups. As increased grooming is associated with an increase in anxiety, this result suggests that as the dose of OLA increases, frequency of grooming increases and therefore it appears to have an anxiogenic effect for males (see Figure 7). For females, animals treated with a low dose of OLA groomed significantly more frequently than any other treatment group, suggesting that this group exhibited the most anxiety-related behavior. Medium dose females groomed significantly less than low dose females but significantly more than high dose females, suggesting that as the dose of OLA increases, grooming behaviour decreases, indicating an anxiolytic effect of OLA. However, with respect to control females, low and medium doses of OLA appear to have an anxiogenic effect, increasing anxiety-related behaviour. Control and low dose females groomed significantly more than their male counterparts, suggesting that, in general, females are more anxious than males for these groups only. Overall, OLA appears to have an anxiogenic effect on males and females with respect to controls, however grooming decreases with an increased dose of OLA. This is an interesting result as, in general, females are seen to be less anxious than males. This reversal in anxious behaviour may be a result of OLA and its anxiogenic effect on females. As seen for the zero maze and rearing in the open field, OLA appears to increase anxiety, especially in females.

Summary

There were overall mixed findings with regards to the effect of dose on anxiety. Firstly, for some measures, olanzapine was associated with an anxiolytic effect. Light occupancy in the light-dark box and over time or with age for rearing and corner occupancy in the open field all showed evidence of an anxiolytic effect of olanzapine. Secondly, however, for other measures, olanzapine was associated with an anxiogenic effect. Open arm observations in the zero maze

decreased with a higher dose of OLA and grooming also increased as olanzapine dose increased, suggesting that olanzapine increased anxious behaviours for these measures. Thirdly, both rearing and corner occupancy showed both anxiogenic and anxiolytic effects. In general for rearing, OLA appears to be initially anxiogenic. Female controls reared more than male controls, suggesting that males were more anxious at baseline, however males treated with a high dose of OLA were seen to engage in rearing more often than females in the same treatment group, suggesting an anxiolytic effect of OLA for males but not females. In the second test, however, rearing reduced only for control animals, suggesting a protective factor of OLA. In terms of corner occupancy, while similarly initially anxiogenic, olanzapine appears to have an anxiolytic effect over time, as the dose increases, anxiety decreases.

4.2.4.2 Locomotor activity

As illustrated in Figure 3, it was found that in the first test, control animals showed significantly higher levels of ambulation than all drug-treated animals, however, there were no significant differences between any of the specific doses of OLA. This suggests that olanzapine does significantly reduce ambulation, but it is not clear to what extent different doses of OLA impact this reduction. However, in the second test, control animals exhibited significantly less locomotor activity than rats in the medium and high dose groups, indicating that while there was no significant change in the drug treatment groups between tests, locomotor activity had significantly reduced for control animals. Rats in the medium dose group were significantly more active than low and high dose treated rats. This suggests that an olanzapine-related effect on locomotor activity had become evident over time or with age.

This lack of olanzapine-related effect between test 1 and test 2 for drug-treated animals may be a result of OLA use, which appears to have a similar effect on locomotor

activity over time or with age. Research by Seliger (1977) in which 50, 100 and 150 day old rats were tested in either a black or white open field found that, while defecation scores remained the same over both trials, ambulation scores decreased for all animals. It is possible that this negative effect of total ambulation scores as seen in the first test may also be due to olanzapine-related locomotor impairment in rats, mice and zebrafish, which has been reported in several studies (Hillebrand et al., 2005; Duncan et al., 2006; Giacomini et al., 2006; Stefanidis et al., 2009; Evers et al., 2010; van der Zwaal et al., 2010; Zhang et al., 2014). Comparable results have also been found in humans (Callaghan et al., 1997; Putzhammer et al., 2005; Roerig et al., 2005). This is common with all antipsychotic medications including SGAs due to their effect on brain dopamine neurons. While these effects are less severe for SGAs compared to conventional antipsychotics such as haloperidol, they are still apparent (Tran et al., 1997; Leucht et al., 1999). Destruction of brain dopaminergic neurons causes Parkinson's disease, the most common movement disorder characterised by bradykinesia, tremor, rigidity and postural imbalance. Olanzapine has exhibited a range of receptor affinities including those for dopamine D1-5 receptors. Olanzapine has also been found to worsen motor symptoms of Parkinson's disease (Molho & Factor, 1999) and reduce cocaine- and dizocilpine-related hyperlocomotion (Meil & Schechter, 1997; Ninan & Kulkarni, 1999). Reduced locomotor activity as a result of olanzapine use may also be one possible explanation as to why SGAs, in particular olanzapine, have such a profound effect on weight gain (van der Zwaal et al., 2010; Evers et al., 2010; van der Zwaal et al., 2012). It is possible that, in terms of the current study, locomotor activity deteriorate for control animals in the second test due to a learning effect in which the previous experience rats had gained in this measure may in turn decrease their associated desire to re-explore their surroundings and therefore reduce exploratory activity. If

this is the case for control animals, it is interesting that ambulation scores for drug treated animals in the second test did not follow this trend and also differ significantly.

4.2.4 Y-maze

In the first test, when first placed in the Y maze, it was found that for female rats, olanzapine does not have a significant effect on the frequency of first choosing the novel arm as none of the treatment groups are significantly different from controls, however the female rats treated with a low dose of OLA chose the novel arm significantly more often than high dose females, but not more than any other group (see Figure 8). This indicates that a higher dose of OLA may be associated with impairment in spatial working memory when compared to a low dose of OLA for females; however this is not significantly different from controls so it did not show a significant effect of olanzapine. A similar result was found for males in the first test. There was no significant effect of olanzapine for any dose with relation to control animals; however male rats treated with a medium dose of olanzapine first chose to enter the novel arm significantly more often than rats treated with a low dose of olanzapine. This suggests the opposite. That is, that males may need a higher dose of the drug for therapeutic efficacy, but this does not result in adverse effects. Females treated with a low dose of OLA chose the novel arm significantly more often than males in the same group, suggesting that males treated with a low dose of OLA have poorer memory recall than female rats based on their capacity to remember the novel arm. In agreement with the previous statements, this suggests that a lower dose of OLA is more beneficial for females but not males. However, this result was found for this group only. In the second test, males treated with both a low and a high dose of OLA entered the novel arm first significantly more often than control males, but not males in the medium-dose group (see Figure 9). This suggests that olanzapine treatment has a positive effect on spatial working memory as males in these groups were

better able to remember which arm was novel compared to control animals. There was no significant result for females in this test.

As seen in Figure 10, there was a significant decrease in frequency of first choosing the novel arm first between the first and second tests for control males and males treated with a medium dose of OLA. This suggests a reduction in ability to recognise the novel arm first. The reverse was true for males treated with a low dose and females treated with a high dose of OLA, whose ability to recognise the novel arm upon first entering the Y maze increase. This indicates that for control and medium dose males, there may be a practice effect associated with the Y maze, or that there is an olanzapine-related negative effect on spatial working memory, but for low dose males and high dose females, olanzapine appears to improve spatial working memory over time or with age.

Overall, this finding suggests that there is a both a positive and negative dose-dependent effect of olanzapine on spatial working memory, however these findings are very mixed making it difficult for conclusions to be drawn. There are contradictory findings regarding the effect of olanzapine on spatial working memory. Skarsfeldt (1996) found that olanzapine did indeed disrupt learning in the Morris water maze and Ortega-Alvaro (2006) also found a negative effect of olanzapine on working memory in the radial arm maze, while Rosengarten & Quartermain (2002) found that olanzapine had no effect on learning processes. A study by Terry et al. (2002) found that after a 4 day washout period, exposure to olanzapine for 45 days did not produce any significant effects on spatial learning, however after a 90-day administration, a persistent impairment in the water maze task was observed. As increases in dopamine promote working memory performance and dopamine agonists facilitate working memory, it makes sense to assume that dopamine receptor antagonists,

such as olanzapine, may impair working memory performance. However, it is possible that the olanzapine-related impairment in working memory could be due to the sedative effects of SGAs (Ortega-Alvaro, 2006).

4.3 Sex effects

4.3.1 Light-dark Box

In the light-dark box, males took significantly longer to emerge into the light compartment compared to females. Longer first emergence latencies suggest higher levels of anxiety (Braun et al., 2011). As rats feel comfortable in enclosed spaces, rapid entry into the light compartment which is generally avoided by anxious rats indicates that females were, overall, less anxious on this measure than male rats. This result is consistent with Archer (1975) who also found that female rats and hamsters emerge sooner into a novel or exposed environment compared to males.

4.3.2 Open Field

Anxiety

In the open field, it was found that males spent significantly more time occupying corner squares than females. This also illustrates the higher levels of anxiety exhibited by male rats in this test of anxiety, as they showed a preference for enclosed spaces. The more time spent occupying corner squares of the open field indicates an avoidance of open spaces and is therefore consistent with the behaviour of an anxious rat. Furthermore, it was also found that males defecated significantly more often than females in the open field. This is also indicative of increased emotionality and anxiety in male rats (Walsh & Cummins, 1976). The combination of decreased movement and increased defecation in males in the open field is thought to represent a frightened or fearful animal. Females are consistently shown to be

less emotional because of their lower rates of defecation and elevated ambulation scores (Meunier & Fischer, 1985) and this study shows consistent results.

Overall, it appears that males were more anxious than females in several measures of anxiety based on their innate preference for familiar, enclosed places. Female rats were observed significantly more often than male rats in open arms of the zero maze, they emerged significantly faster into the open compartment of the light-dark box, and spent less time in the corner squares of the open field. Male rats also defecated significantly more than female rats. This is consistent with several studies which report the increased emotionality of males compared to females, and that females appear to be more active and less anxious than male rats (Gray, 1971; Archer, 1973, 1975). However, in the open field, generally, male rats were observed to engage in grooming significantly less often than female rats. As an increase in grooming is also associated with an increase in anxiety levels, this result suggests the opposite. That is, that female rats appeared to be more anxious than males for this measure (Walsh & Cummins, 1976; Diaz-Moran et al., 2014). This appears to be due to due to the effect of OLA.

Locomotor activity

With regard to the effect of sex on locomotor activity, females were observed to have significantly higher levels of ambulation in the open field compared to males. This result is consistent with several studies (Archer, 1975; Seliger, 1977; Meunier & Fishcer, 1985; Slob et al., 1986) who all found increased ambulation in females compared to males. This increased locomotor activity may also be an index of an increase in exploratory behaviour, and a decrease in anxiety in female rats. Percentage of entries and transitions in the zero maze, Y maze and the light-dark box are also measures of ambulation, but were not

significant when assessing sex differences. This increased locomotor activity may also be an index of an increase in exploratory behaviour, and a decrease in anxiety in female rats, consistent with the reduced anxiety-like behaviour in female as opposed to male rats.

4.3.3 Y-maze

In terms of the effect of sex on total arm entries in the Y maze, in the first test, female rats made significantly more total arm entries than male rats. Although this does not suggest a learning deficit, it does suggest that female rats showed increased locomotor activity and perhaps a reduction in anxiety, which is consistent with previous findings in the current study, and previous research stating that females appear to be more active and less anxious than male rats (Gray, 1971; Archer, 1973, 1975; Seliger, 1977; Slob et al., 1986).

4.4 Test/age effects

4.4.1 Open Field

A significant main effect of test for ambulation in the open field showed that all subjects ambulated significantly more often in the first test than in the second. However, this result is more appropriately explained in terms of a significant dose x test interaction, as described above.

A main effect of test for grooming in the open field was also found. Grooming significantly decreased between test 1 and test 2, suggesting that there is a decrease in anxiety in the open field between these two tests. Furthermore, this indicates an anxiolytic effect of OLA as this anxiety-related behaviour decreases over time or with age.

4.5 Limitations

There are a number of limitations that have become clear upon the completion of this study that are worth mentioning. Firstly, a histological analysis of the rats' brains was not performed upon the completion of the experiment. This would have provided greater insight into the structural and chemical changes as a result of olanzapine treatment and how this differed based on the sex of the animal, such as dopamine and serotonin. Changes in serotonin could account for the anxiety, memory or locomotor effects found in this experiment as it is implicated in each of these functions (Graeff et al., 1996; Karl et al., 2006; Locchi et al., 2008). As both dopamine and serotonin levels are altered by olanzapine, this analysis could reveal some interesting and important changes. However, the focus of this thesis was not on anatomical and neurochemical changes but on behavioural measures.

Secondly, long-term effects of olanzapine use were not examined. This is an important limitation as it is possible that humans may be prescribed SGAs such as olanzapine in the long term, and effects seen in the present study may only be preliminary when considering the long-term use of the drug. Furthermore, it is important to investigate possible sex differences associated with long term use as any differences or the extent of the differences may change over the course of several years. However, because this study focused on the effects in early adulthood, this limitation is not a significant exclusion.

Thirdly, observer reliability tests were not used to ensure all observations were consistent. It is possible for an observer or in this case, the experimenter, to subconsciously observe what they believe they should be seeing while observing the animals' behaviour, rather observe than what is truly occurring (Kaufman & Rosenthal, 2009). Therefore, it is possible that mistakes were made during the experiment as notation was made every 3

seconds, and this fast pace may have contributed to mistakes in what the researcher thought they had seen and thus, what was recorded and analysed (Kaufman & Rosenthal, 2009). This may result in a question as to whether results recorded were in fact reliable. In order to get around this limitation, it is suggested that the use of a second observer would be useful to conduct inter-observer reliability tests to assess the reliability of the experimenter and ensure all recorded results were accurate (Kaufman & Rosenthal, 2009). However, this was not included in the current study. Even though this is a limitation, it does, however, mean that a single experimenter would be consistent throughout the entirety of the experiment.

Lastly, extraneous variables related to the room in which animals were tested is a limitation of the current study. It is important to note that the timing of the experiments varied from day to day due to time constraints and other commitments of the experimenter. Some animals were tested early in the morning and others late at night. The timing of the testing is an important limitation as it can affect locomotion and anxiety responses (Kaya, Karakaş, & Coşkun, 2011). However, these variables were not always present and therefore they may not have had a strong enough implication on the results. Furthermore, other rats which were not tested were in the same room as the rat being tested and it is possible that the smell of the other rats could have directed the attention of the animals elsewhere. Steps were taken to separate rats as much as possible to reduce this issue.

4.6 Future directions

The results of this study highlight the fundamental differences between males and females in their responses to anxiety, locomotor activity and spatial working memory, and the effects of olanzapine on these. There are several differences in sex, dose and the age at which animals were tested that suggest that these differences need to be addressed to reduce the risk

and increase the benefit associated with SGAs. The current study illustrates some important facts that will require additional research. Firstly, the limitations of this study need to be addressed. A histological analysis would be useful to identify brain changes as a result of olanzapine exposure and provide a more comprehensive understanding of sex differences in olanzapine treatment and how this may explain behaviour. It is also suggested that the time frame in which animals were exposed to olanzapine be lengthened to investigate sex differences and chronic exposure, and whether the age of the animals plays an important role. Future research should focus on replication of the current experiment to ensure accurate, reliable and generalisable findings, perhaps non-inclusive of the test factor in order to avoid any issues that may be correlated with learning effects upon re-exposure of rats to the different types of apparatus.

4.7 Conclusions

The aim of this experiment was to examine whether there were any sex differences in the behaviours exhibited by male and female rats after acute exposure to the commonly used atypical antipsychotic olanzapine. This study also aimed to clarify the issue surrounding whether atypical antipsychotics are anxiolytic, anxiogenic or have no effect on anxiety, and to determine the extent to which SGA-related impairments in locomotor activity and spatial working memory differ dependent on the sex of the animal. It can be concluded that there are marked differences in the behavioural responses to olanzapine in males and females. Although the exact nature of some of these differences is unclear at present, the fact that they exist is obvious. For example, olanzapine elicited a clear anxiogenic effect for females in the zero maze, however effects on males were unclear and females appeared to experience anxiety-like symptoms at higher doses that were significantly different to males when looking at rearing in the open field. There is strong evidence to suggest that olanzapine has an

anxiogenic effect on anxiety-related behaviours, however anxiolytic effects are still apparent, as highlighted in the light-dark box and in the open field for rearing, corner occupancy and grooming. Nevertheless, it is clear that it does have an effect on anxiety-related behaviour. Olanzapine also has an overall negative effect on locomotor activity over time, though this effect is dose dependent. With regard to spatial working memory, there are also considerably contradictory findings, with the suggestion of a negative effect of olanzapine on memory over time, however it appears that females may be more resilient to this effect than males. Overall, it can be concluded that the response of males and females to olanzapine differs in many cases. These results highlight these marked differences and suggest that a dose that may be safe and efficacious for males may not be the same dose that is safe and efficacious for females. Behavioural responses in anxiety, locomotor and working memory tasks varied dependent on the sex of the animal. These results may have implications for the use of the drug with humans as it has been proven that females do experience significantly more adverse effects of the drug with respect to males. This may be a result of the given dose of the drug; reducing the dose for female patients may result in an improved prognosis, with fewer adverse effects and a better efficacy. It is therefore recommended that further research be undertaken to determine the extent to which these differences may have an impact on the safety and efficacy of olanzapine as a prescription medication for women, as well as men.

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6 Appendix A

UNIVERSITY OF CANTERBURY
Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

ANIMAL ETHICS COMMITTEE

Secretary, Rebecca Robinson
Telephone: +64 03 369 4588, Extn 94588
Email: animal-ethics@canterbury.ac.nz

Ref: 2017/09R

26 April 2017

Molly Lockington
Psychology
UNIVERISTY OF CANTERBURY

Dear Molly

I am pleased to inform you that the Animal Ethics Committee (AEC) has approved your application entitled: The impact of sex differences on the behavioural effects of olanzapine.

Approval has been granted:
(a) for the use of 80 PVG/c hooded rats.
(b) for your research project to be undertaken from 19 April 2017 to 19 October 2017. If you require an extension of this period please contact the AEC Secretary.

As part of AEC's new Code of Ethical Conduct all applicants receiving approval to work on animals are required to provide a final report at the completion of their project. The purpose is to provide the AEC with a record of your use of animals and what was achieved by your research project. We are very much interested in your findings and to learn what you have achieved. Following the completion date indicated above you are asked to provide this report using the new Final Report form which is available at the AEC web site (https://intranet.canterbury.ac.nz/research/ethics.shtml).

On an annual basis the University is legally required to provide to MPI statistical data on all animal manipulations undertaken in a calendar year. To assist us in collating this information you are also required to complete and return to the AEC Secretary the attached MPI Animal Manipulation Statistical form 30 days after the completion of this project, or once every three years, whichever comes first. If no animals have been manipulated in your project please provide a "Nil" return. Please also find enclosed a copy of the Animal Welfare (Records and Statistics) Regulations 1999 for your information, together with a list of Animal Type Codes and brief guideline notes for your assistance.

Yours sincerely

James Briskie

Associate Professor Jim Briskie
Chair
University of Canterbury Animal Ethics Committee