Effectiveness of Cannabidiol in reducing Ketamine-induced schizophrenia-like behaviour in both male and female rats

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Abstract

Schizophrenia is a debilitating and costly mental illness. Many patients do not respond well to currently available treatments, and adverse side effects are common. Cannabidiol (CBD), a natural component of the *Cannabis Sativa* plant, has been shown to have a number of therapeutic qualities, including potential as a new antipsychotic. Although CBD has been used in several different models of schizophrenia, previous research has failed to consider possible sex differences in responsiveness to the compound. The present research therefore used both male and female rats in the widely used ketamine model of schizophrenia. PVG/C Hooded rats were randomly assigned to one of four experimental conditions: a saline only control group (saline injection followed by second saline injection; N = 6M, 6F); ketamine only group (ketamine injection followed by saline injection; N = 6M, 6F); cannabidiol low dose group (ketamine injection followed by a cannabidiol injection of 10mg/kg; N = 6M, 6F); and a cannabidiol high dose group (ketamine injection followed by a cannabidiol injection of 20mg/kg; N = 6M, 6F). Behavioural testing occurred in a Y-maze and open-field, where both normal and stereotyped behaviours were recorded, as well as locomotor activity and spatial memory. Ketamine successfully induced stereotypy but failed to induce hyperlocomotion. Findings support the potential antipsychotic effects of CBD, particularly for reducing stereotypic behaviour in females. Results found data trends that suggest sex differences in responsiveness to CBD when administered with ketamine, although further research is needed due to lack of statistical significance.

1.0 Introduction

1.1 General Overview

The general aim of this research was to further investigate the antipsychotic properties of cannabidiol (CBD). This research also aimed to contribute to the increasing body of literature surrounding this topic. The following introduction discusses mental illness generally, before moving on to schizophrenia specifically, and its currently available treatments. The various hypotheses of the aetiology of schizophrenia will also be considered, with an emphasis on the glutamate hypothesis and NMDA receptor antagonists**.** Several animal models of schizophrenic-like symptoms will be outlined. Cannabinoids - with specific reference to cannabidiol - will be examined, and research into cannabidiol as an antipsychotic will be discussed before outlining the specific aims and hypotheses of the present study.

Approximately 1 in 5 (20.7%) New Zealanders will have experienced mental illness at some point in the last 12 months, with 46.7% of the population meeting criteria for a disorder at some point in their lifetime (Oakley Browne, Wells, & Scotts, 2006). Due to national public awareness campaigns aiming to reduce stigma and discrimination (Ministry of Health, 2007) society is becoming more aware about mental illness. However, the individuals affected can still struggle with discrimination from much of society, and this can influence the impact that mental illness has on the individual in terms of every day functioning and their recovery. A study by Peterson and colleagues stated that individuals with mental illness reported discrimination most frequently by friends and family (59%) and when searching for employment (34%) (Peterson, Pere, Sheehan, & Surgenor, 2007). Effective treatments are immensely important for these individuals, as is support to maximise quality of life.

The costs of mental illness in this country and internationally, both short and long term are huge. To take one amongst a large number of examples: an overrepresented proportion of prison inmates in New Zealand suffer from a mental illness (with inmates having approximately twice the rate of most illnesses than the general population), and many of these go untreated. For example, 63% of inmates suffering from psychosis fail to receive treatment, as do 53.6% with major depression and 58.6% suffering from posttraumatic stress disorder (Brinded, Simpson, Laidlaw, Fairley, & Malcolm, 2001). This can lead to the situation where an incarcerated individual who suffers from mental illness will be released from prison back in to the community, and in the absence of proper treatment and support, that individual may rapidly be caught back up in the legal system, and be re-incarcerated. This so-called 'revolving door' phenomenon has significant costs for all parties involved, including local and wider communities. Not only is it vital for the individuals who suffer from illness to be able to have access to a treatment that works for them, but is also important for society as whole.

Schizophrenia, with its combination of symptoms ranging from cognitive impairments and social withdrawal to hallucinations and delusions, is the most debilitating and costly mental illness (Combs & Muester, 2007). Affecting approximately 1% of the global population (Furuta & Kunugi, 2008; Julien, 2008), a range of effective treatments is required. As will be discussed, research in to the illness, its aetiology and treatments have come a long way, but with so many individuals who do not respond well to conventional medications and the side effects that often accompany them, researchers continue to strive to understand more about the illness and how to treat it.

1.2 Schizophrenia

Schizophrenia is a mental illness that presents itself with a range of both positive and negative symptoms, as well as cognitive impairments such as deficits in memory, learning and attention. Schizophrenia was first recognised as a disorder in the early 1900s by Kraepelin, who named the illness 'dementia praecox' after observing a number of symptoms including paranoia and emotional blunting in patients (Andreasen, 1997). Although historically there has been some debate surrounding what symptoms should be included in schizophrenia diagnosis, it is undoubtedly characterised by deficits in social functioning, difficulties in looking after oneself, and intellectual impairments (Combs & Muester, 2007). These symptoms are currently necessary criteria in most current systems for diagnosis (Combs & Muester, 2007).

Due to the effects of these symptoms, individuals diagnosed with schizophrenia often fail to remain independent and stay in their own homes. It has been reported that a substantial percent (25-60%) of patients rely on relatives as caregivers, and others often end up in mental health institutions and residential units. A study in the United States of America found that many individuals who fall through the system may end up in jail, with rates of the mentally ill in jails rising back up to that of the 1800's, when it was common practice for those with mental illness to be incarcerated. Currently 7.2% of prison inmates suffer from serious mental illness, many of whom are being held without criminal charges having been laid against them (Torrey et al., 1992).

Schizophrenia onset usually occurs around late adolescence and early adulthood. Childhood onset may occur, although this is less likely (Asarnow & Asarnow, 2003) and there is some debate as to whether or not this is the same disorder (Combs & Muester, 2007).

It has also been shown that women tend to develop schizophrenia later in life than men. It has been suggested that this may be attributed to menopause (Lees, Hallak, Deakin, & Dursun, 2004) and therefore, women may be protected to some extent from developing schizophrenia due to the protective qualities of oestrogen. Females also present with more positive symptoms than men, and men with more negative symptoms than women (Lees, et al., 2004).

The positive symptoms of schizophrenia "are noted by an excess or distortion of normal behaviour or cognition (e.g., hallucinations and delusions), and are usually a distressing experience for the client" (Rollins, Bond, Lysaker, McGrew, & Salyers, 2010, p. 209). These symptoms are often episodic and the rapid onset contributes to the disturbance of everyday life. Three major positive symptoms that individuals present with are hallucinations, delusions and disorganised behaviour. Hallucinations occur in about 75% of individuals with schizophrenia (Combs & Muester, 2007) and can be auditory, visual, olfactory or tactile. The most common of these is auditory hallucinations, occurring in 74% of patients (Sartorius, Shapiro, & Jablensky, 1974). The prevalence of visual hallucinations is much lower (10-15%, Combs & Muester, 2007). Delusions may be bizarre (e.g. that the person can control thought), but are most commonly persecutory (e.g. imagining that people are trying to harm them) with persecutory delusions occurring in 64% of patients (Sartorius, et al., 1974). Other positive symptoms include stereotyped behaviour. In patients diagnosed with schizophrenia, stereotypy presents itself as repetitive and functionless motor behaviours (Morrens, Hulstijn, Lewi, De Hert, & Sabbe, 2006). Little is known about the course of stereotypy in schizophrenia, unlike cognitive symptoms which are relatively stable.

The negative symptoms of schizophrenia are considered to be the lack or absence of normal human qualities, such as the loss of motivation, the blunting of emotions, poverty of

speech, lack of pleasure and social withdrawal (Julien, 2008; Rollins, et al., 2010). These symptoms are likely to be stable and continue for the course of the illness (Rollins, et al., 2010). Similarly, cognitive symptoms can be ongoing. Symptoms include deficits in learning, working memory, attention and processing information, and difficulties in abstract reasoning (Combs & Muester, 2007). In the late 1960s, an international study in to schizophrenia conducted by Sartorius and colleagues reported that 97% of patients had a lack of insight (Sartorius, et al., 1974). These symptoms play a significant role in the degree to which everyday tasks become too difficult for the individual (Leifker, Bowie, & Harvey, 2009).

Depending on the types of symptoms displayed, schizophrenia is referred to as paranoid, disorganised, catatonic, residual or undifferentiated (Barlow & Durand, 2005). Diagnosis in to these subtypes is dependent on which symptom criteria the individual meets. Comorbidity often occurs with negative mood, depression, and substance abuse in adults (Combs & Muester, 2007), and conduct disorders and depression in children (Asarnow & Asarnow, 2003). Due to low mood, suicide rates are high in adults (Combs & Muester, 2007).

These symptoms all have a massive impact on the quality of life of the individual (Hoffmann, Kupper, & Kunz, 2000). A study of the relationship between symptoms and life outcomes for residential patients found that hallucinations, suspiciousness and social skills all predicted functioning outcomes. Furthermore, all predictive variables (including symptoms) only accounted for 40% of the variance, suggesting that symptoms aside, other environmental factors contribute to everyday functioning (Leifker, et al., 2009).

Using data from the Mental Health Information National Collection (MHINC), Kake and colleagues estimated that there is a higher prevalence of schizophrenia for Maori than for

non-Maori. These estimates took into consideration socioeconomic deprivation and age. It has been reported that adolescent Maori males have a higher prevalence of cannabis use. Since the use of cannabis has been related to the development of schizophrenia, this may explain some of the increased prevalence in Maori (Kake, Arnold, & Ellis, 2008). Another study also found differences in treatments used for various ethnic groups in New Zealand, including Maori and Pacific people. It has been suggested that differences in metabolism may be important between ethnic groups and that some ethnic groups may be more sensitive to certain receptors, meaning they respond differently to various pharmacological treatments (Wheeler, Humberstone, & Robinson, 2008). This again demonstrates the importance for a variety of treatments, as not all individuals respond to the same medications.

1.2.1 Treatment

Some individuals who suffer from schizophrenia do not benefit from conventional medications and treatments. Further understanding of potential treatments for decreasing schizophrenic symptoms is valuable to those individuals and families affected by the illness, and to those involved in the mental health sector and the wider community.

Before the discovery of antipsychotic agents, patients with schizophrenia were ostracised from society and many were permanently hospitalised, or as previously noted, prior to the 19th century, even jailed (Torrey, et al., 1992). In the 1930's electroconvulsive therapy was used, which often caused broken bones for the patient, and in the 1940's, prefrontal leucotomies (the destroying of the frontal cortex with a blunt knife) was common (Rosenbloom, 2002). The development of medications that alleviate symptoms has allowed many individuals with schizophrenia to lead typical lives, when previously they almost certainly would not have this opportunity. Although a number of medications are now

available, many of them have side effects which cause patients to stop taking them when their symptoms improve. This often leads to a relapse of symptoms and deterioration in functioning, and sometimes even rehospitalisation. Research into novel and effective medications with fewer side effects is ongoing and continuously needed.

In the mid 1950s, phenothiazines were discovered, and although the drugs were first developed as antihistamines, the discovery of their sedative properties soon followed. After the use of phenothiazines such as promethazine and chlorpromazine by surgeons and anaesthesiologists, French research psychiatrists Delay and Deniker studied the effects of chlorpromazine on schizophrenic patients (Delay & Deniker, 1955). The researchers found the drug to be successful in alleviating mania and psychotic symptoms (Rosenbloom, 2002). Following this breakthrough, a number of other drugs with fewer side effects were developed over time, starting with analogues of the phenothiazines (Julien, 2008; Mailman & Murthy, 2010).

Currently, a variety of antipsychotics are available, which are categorised on the basis of when they were discovered. The first-generation or "typical" antipsychotics, such as those mentioned above, are effective in decreasing positive symptoms, but are often accompanied by a range of extrapyramidial side-effects (EPS) such as movement disorders. Typical antipsychotics can also exacerbate or even induce negative and cognitive symptoms in the patient (Keltner & Johnson, 2002). Both the clinical efficacy and the EPS of these antipsychotics, are a result of their dopamine D2 receptor antagonism (Julien, 2008; Keltner & Johnson, 2002).

In the late 1980s, a second generation of antipsychotics appeared. These second generation or "atypical" antipsychotics are typically preferred for treatment as they do not cause EPS, although other side-effects such as weight gain have been associated with them. Atypical antipsychotics are more effective than typical antipsychotics in treating negative symptoms. This has been attributed to their blocking of serotonin 5-HT2 receptors, as well as their D2 receptor antagonism (Julien, 2008; Keltner & Johnson, 2002). However, this explanation has also been disputed, reasons for which will be discussed below (Kapur & Remington, 2001).

It is now thought that there may be a new third-generation of antipsychotics, although at present, most are still described as either "typical" or "atypical". Aripiprazole, a recently available antipsychotic, and is thought to be in this category. What sets this drug apart from earlier antipsychotics is that it is a partial D2 receptor agonist, and this is thought to allow it to stabilise the dopamine system (Keltner & Johnson, 2002; Mailman & Murthy, 2010). It is also worth noting that atypical antipsychotics are now used for other various disorders such as bipolar disorder, personality disorders, and dementia (Julien, 2008). The implications of discovering novel drugs that may work for a variety of people and have minimal side effects are immeasurable.

1.2.2 Aetiology of Schizophrenia

Although the causes of schizophrenia are not entirely understood, various factors that include biological factors, stress, sex, ethnicity, structural brain abnormalities and psychosocial deficiencies have been linked to the illness. As previously noted, there are sex and ethnic differences in the prevalence of schizophrenia, and potentially in the effectiveness

of certain treatments. It has also been hypothesised that stress may contribute to the development of schizophrenia, particularly in individuals who may be vulnerable (e.g. have a genetic predisposition). There are a number of areas of the brain that are thought to be implicated in the aetiology of schizophrenia. Consistent findings from clinical and post-mortem research show that the hippocampus has a significantly lower volume in patients that have been diagnosed with schizophrenia than controls (Keilhoff, Bernstein, Becker, Grecksch, & Wolf, 2004). It has also been widely shown that patients with schizophrenia have a significantly lower overall brain volume. These abnormalities are thought to occur during foetal development (Asarnow & Asarnow, 2003).

It has been widely recognised that schizophrenia appears to be highly hereditary (Nieratschker, Nöthen, & Rietschel, 2010). This finding has sparked debate of whether this is due to biological factors or learned behaviour. Adoption and twin studies have been important in this respect, and have supported the role of genetics. Although having a parent with schizophrenia increases the risk of developing the illness, it is not inevitable (Combs & Muester, 2007). This is demonstrated by the fact that even in identical twins, who share 100% of the same genes, one may develop the illness and the other may not. The implication of this is to suggest an interaction between genetic vulnerability and environment. While these are all considered to be contributing factors to the aetiology of schizophrenia, a neurochemical component is still sought (Emrich, Leweke, & Schneider, 1997).

A number of models have been developed around the changes observed in various neurotransmitter systems in patients diagnosed with schizophrenia. As can be seen above, the majority of antipsychotics have been considered in terms of their actions on the dopamine system, and this is where the focus of much research into novel antipsychotics has been

directed. However, as more research is conducted on the pharmacological basis of schizophrenia, more models and hypotheses of the illness' development are emerging.

1.2.2.1 Dopamine Hypothesis

There is still wide acceptance of the dopamine hypothesis of schizophrenia, which was the dominant theory of the aetiology of the illness for decades (Haracz, 1982; Itokawa, Arinami, & Toru, 2010; Matthysse, 1974). This hypothesis posits that an excess release of dopamine or a possible heightened sensitivity to dopamine receptor neurons is the cause of the positive symptoms associated with schizophrenia (Mailman & Murthy, 2010).

The involvement of dopamine was first suggested in the 1970's after a number of early studies found that current neuroleptics were associated with changes in dopamine. Studies that tested the cerebrospinal fluid of schizophrenic patients attributed changes in brain dopamine to be due to the administration of chlorpromazine, clozapine and haloperidol (Gerlach, Koppelhus, Helweg, & Monrad, 1974). Another study involving cats found that the administration of L-Dopa (the precursor of dopamine) induced a range of stereotypic behaviours, which provided evidence that an excess of dopamine, or hypersensitivity to it, may have been related to schizophrenic disorders (Stevens, Wilson, & Foote, 1974). An early review by Matthysse concluded that the bulk of evidence available at the time supported the dopamine hypothesis, although there were a number of limitations and contradictions that were, at the time, yet to be resolved (Fyro, Wode-Helgodt, Borg, & Sedvall, 1974; Matthysse, 1974).

Although many still strongly support this hypothesis, there are those who are moving towards other explanations for the dopamine abnormalities seen in patients with schizophrenia. Moncrieff concluded that contradictory results from post-mortem studies showed that schizophrenic symptoms being caused by over activity of dopamine are in fact not supported by current evidence. The author then suggested that dopamine antagonists may only reduce the intensity of symptoms, rather than target underlying mechanisms of the illness (Moncrieff, 2009). It has also been suggested that dopamine abnormalities are actually the result of abnormalities that lie in other neurotransmitter systems (see section 1.2.2.4).

1.2.2.2 Serotonin Hypothesis

The serotonin system is also believed to be involved in schizophrenia aetiology. During the development of atypical antipsychotics, improvements in patients were hypothesised to be due to 5-HT2 receptor antagonism (Julien, 2008). As noted previously, the notion that serotonin is responsible for the improvements seen by use of atypical versus typical antipsychotics have been disputed. This is mainly due to the fact that, as well as atypical antipsychotics, some typical antipsychotics have a high affinity for serotonin receptors, and that some substances that have a high affinity for serotonin receptors alone are not able to help with schizophrenia symptoms (Kapur & Remington, 2001). There is also evidence that supports an interaction between dopamine and serotonin. However, Meltzer emphasised that since atypical antipsychotics are able to control psychotic symptoms better than typical antipsychotics and alleviate extra-pyramidal side effects, serotonin antagonism is important in schizophrenia aetiology (Meltzer, 1992). Thus, although there seems to be a role for serotonin, this role is not entirely clear

1.2.2.3 Cannabinoid Hypothesis

It has been suggested that the endogenous cannabinoid system may also be involved. It was observed very early on that the effects of the *Cannabis sativa* plant, when consumed, closely resembled the symptoms of schizophrenia. A substantial body of research has used *Cannabis sativa* or its main active constituent, Δ9-tetrahydrocannabinol (THC), to model psychosis in humans (Koethe, Hoyer, & Leweke, 2009). For example, D'Souza and colleagues gave healthy participants either 2.5 or 5mg/kg of THC, or ethanol vehicle alone. Participants were tested for positive and negative symptoms, alterations in perception, and learning and recall. The authors found that THC induced positive and negative symptoms, significantly impaired recall and working memory, but surprisingly did not alter learning (D'Souza et al., 2004). In a subsequent study, the above methods were replicated with stable schizophrenic patients. Amongst their findings, the authors reported that THC induced the same symptoms listed above, and to a higher extent than in the healthy participants (D'Souza et al., 2005).

It is thought that irregularities in the functions of cannabinoid receptors, particularly the cannabinoid CB_1 receptor, may be involved in schizophrenia. Human studies have found dysfunction in the CB_1 receptor in schizophrenia patients, and these abnormalities are thought to be involved in cognitive and memory impairment (Parolaro, Realini, Vigano, Guidali, & Rubino, 2010). CB_1 receptor antagonists (such as cannabidiol) have also been shown to attenuate pharmacological psychosis in various animal models of schizophrenia (Kölfalvi & Fritzsche, 2008). The endocannabinoid system has also been linked to the dopaminergic (Muller-Vahl & Emrich, 2008) and glutamatergic systems (Emrich, et al., 1997). However, the interactions between these systems are not yet understood.

Although the link between schizophrenia and the endocannabinoid system is relatively well established, a cannabinoid hypothesis of schizophrenia is still being developed and is yet to be used as an animal model in much research (Emrich, et al., 1997). This is partially due to the fact that the endocannabinoid system was only discovered in the 1990's, and therefore, there are still gaps in knowledge about the mechanisms of action of cannabinoids (Koethe, et al., 2009). Cannabinoids and the substance cannabidiol will be discussed in more detail below. It is worth noting that other neurotransmitter models have also been suggested such as the cholinergic, GABAergic and peptidergic models (Emrich, et al., 1997). With the hunt for a single cause of schizophrenia now diminished, current research points toward a more complex pattern of causes.

1.2.2.4 Glutamate Hypothesis

A current model that is receiving a great deal of interest is the glutamate hypothesis. Glutamate is the major excitatory neurotransmitter in the brain. There are both ionotropic (NMDA, AMPA and kainite) and metabotropic (mGlu receptors) glutamate receptors (Hansen, Petersen, & Hansen, 2006). It has been suggested that blockade of the ionotropic receptor, *N*-methyl-D-aspartate (NMDA), is responsible for a succession of events that mimic the appearance of psychosis and schizophrenic-like symptoms in both humans and animals (Large, 2007).

The involvement of glutamate in schizophrenia was established in the 1980s after researchers found that there was diminished glutamate in the cerebrospinal fluid of patients with the illness (Kim, Kornhuber, Schmid-Burgk, & Holzmuller, 1980). This suggested that glutamatergic abnormalities may be involved in the aetiology of schizophrenia. Support for this hypothesis includes research which has shown that several features of the illness cannot

be explained by previous hypotheses alone, such as the dopamine hypothesis (Stone, 2009). Since glutamatergic deficiency has been shown to interact with dopamine (Tiedtke, Bischoff, & Schmidt, 1990), the effects that antagonists of the NMDA glutamate receptor can have on this and other neurotransmitter systems may provide a useful model for explaining schizophrenic symptomology. It is thought that since NMDA receptors regulate dopamine neurons, hypofunction of NMDA receptors may be responsible for the abnormal dopamine activity observed in patients with schizophrenia (Stahl, 2007). It has been proposed that this hypofunction of NMDA receptors results from neurodevelopmental abnormalities that occur while the glutamate synapses are forming (Stahl, 2007).

Antagonists of the NMDA glutamate receptor, such as ketamine and phencyclidine (PCP), effect both dopamine as well as glutamate receptors (Seeman, 2009) and can also disrupt the neurochemistry of multiple brain regions (Gao, Elmer, Adams-Huet, & Tamminga, 2009). This may help explain why, unlike dopaminergic or serotonergic drugs of abuse which only induce effects resembling the positive symptoms of schizophrenia, substances such as ketamine and PCP can induce a range of positive and negative schizophrenia-like symptoms, as well as cognitive deficits in both animals and humans (Julien, 2008; Stone, Morrison, & Pilowsky, 2007). This suggests that many symptoms of schizophrenia are likely to be induced by NMDA receptor antagonism (Stone, et al., 2007). These substances can also cause stable schizophrenic patients to relapse (Stone, et al., 2007), and the administration of known antipsychotics can attenuate such induced effects (Bubeníková-Valešová, Horáček, Vrajová, & Höschl, 2008; Marcotte, Pearson, & Srivastava, 2001). Therefore, this model of schizophrenia which is based on the glutamate system is useful for testing the potential of novel antipsychotics.

1.3 NMDA Glutamate Receptor Antagonists

Three of the most studied NDMA receptor antagonists are PCP, MK-801 and ketamine. Both ketamine and PCP are now widely used as animal models of schizophrenia, and there is a growing amount of research on MK-801. These models of schizophrenia are based on our current understandings of the glutamate hypothesis.

By blocking NMDA receptors, PCP and ketamine (both dissociative anaesthetics) can result in behavioural abnormalities when administered in sub-anaesthetic doses (Becker et al., 2003; Wilson et al., 2005). In humans, ketamine and PCP can worsen symptoms or reinstate psychosis in remitted schizophrenic patients. In healthy volunteers, these substances can induce psychotic episodes, cognitive disturbances, hallucinations and alter mood (Breese, Knapp, & Moy, 2002; Lahti, Weiler, Michaelidis, Parwani, & Tamminga, 2001; Stone, et al., 2007). A study by Vollenweider and colleagues found that in healthy males, ketamine produced a dissociative state and schizophrenia-like symptoms (Vollenweider, Vontobel, Øye, Hell, & Leenders, 2000). Similarly, auditory sensory memory is impaired by ketamine (Umbricht et al., 2000).

In animal models, PCP, ketamine and MK-801 induce hyperactivity and stereotypy. MK-801 is reported to induce stereotyped behaviours such as repetitive sniffing, and these effects are delayed and shortened by the antipsychotic haloperidol, and antagonised by clozapine (Tiedtke, et al., 1990). PCP has been reported to disrupt learning and memory in a variety of maze tests (Furuta & Kunugi, 2008) and induce cognitive deficits, which have then been attenuated by antipsychotics such as clozapine (Amitai, Semenova, & Markou, 2007). Although PCP is still readily used in animal studies, it is no longer used in controlled human studies because of its neurotoxicity. Ketamine however, induces similar effects but is less

toxic (Jentsch & Roth, 1999). Because of this, the present research focussed on ketamine as an animal model. The effects of the substance are described in more detail below.

1.4 Animal Models

Although it is impossible to produce an animal model that is a complete reproduction of schizophrenia in humans, a number of current models show certain characteristics of the disorder (Furuta & Kunugi, 2008). These models are crucial for understanding the aetiology and mechanisms of schizophrenia, and are important for developing treatments for the disorder. As previously noted, ketamine has been widely used as an animal model in schizophrenia research due to its NMDA receptor antagonism. The present study therefore focussed on ketamine and previous research involving current models of the behavioural assessment of schizophrenic-like symptoms.

1.4.1 Locomotor Activity

One of the most widely used measures of the drug-induced positive symptoms of schizophrenia is changes in locomotor activity. This is partly due to the fact that locomotor activity can be easily measured and quantified. Locomotor activity is typically measured by recording the number of squares a rodent crosses in an open field thereby providing an indication of the distance travelled. It is believed that this model assesses sensory information processes that may underlie some symptoms of schizophrenia (Geyer & Moghaddam, 2002). With regards to the glutamate model of schizophrenia, it has been widely reported that the administration of NMDA antagonists induces hyperactivity in rodents.

Rodvelt and colleagues administered ketamine to rats daily for 30 days**.** Locomotor activity was assessed at the end of the 30 day period, in an open field. The authors reported

that rats that had received 30-50mg/kg ketamine displayed significantly higher levels of hyperactivity than rats administered saline (Rodvelt, Kracke, Schachtman, & Miller, 2008). Similarly, Hayse and colleagues reported hyperlocomotion in rats 15 minutes after receiving 30mg/kg ketamine. However, rats that received a dose of 100mg/kg displayed hypolomotion 15 minutes later, followed then by hyperlocomotion after 60 minutes (Hayase, Yamamoto, & Yamamoto, 2006). Other authors have also reported a significant increase in locomotor activity in both mice (100mg/kg, Chatterjee, Ganguly, Srivastava, & Palit, 2010) and rats (60mg/kg, Leite, Guimaraes, & Moreira, 2008) for ketamine, MK-801 (0.4mg/kg, Leite, et al., 2008) and PCP (2mg/kg, Sams-Dodd, 1998), thus further supporting this behavioural measure as a model in the glutamate hypothesis.

1.4.2 Cognitive Abnormalities

It is not possible to examine negative symptoms of schizophrenia such as hallucinations and delusions in animals, and other symptoms such as emotional withdrawal and apathy are difficult to model accurately. It is however possible to examine and measure changes in cognitive abnormalities such as learning and memory. NMDA antagonists have been shown to induce learning and memory deficits. Traverso and colleagues found that ketamine doses of 75mg/kg and 120mg/kg disrupted conditioned taste aversion in male rats (another widely used test for learning (Traverso, Ruiz, Camino, & De la Casa, 2008).

Learning and memory can be tested in a variety of ways, usually in a maze test such as the Morris water maze task, radial-arm maze, or T-maze (Furuta & Kunugi, 2008). A widely used measure for learning and memory in rats is the Y maze. This apparatus has been used by Hughes (2001, 2004) for developing a test of responsiveness to brightness change in rats and thus a simple test of short-term spatial memory. By means of these tests, cognitive

changes can be assessed in animals that have been treated with a substance such as ketamine, thereby providing some insight into mechanisms underlying the cognitive changes that occur in schizophrenia.

1.4.3 Stereotyped Behaviours

As discussed previously, stereotypy is a positive symptom that often presents in schizophrenia. It has also been noted that normal individuals administered PCP exhibit stereotyped-like behaviours such as rocking and head shaking which are similar to what patients with schizophrenia may experience (Breese, et al., 2002). These symptoms are also observed in animal models of the illness. In rodent models, behaviours include but are not limited to, sniffing, grooming, reverse locomotion, head weaving, gnawing and circling. Frequencies of stereotyped behaviours in rats are usually observed in open fields or the like.

Randrup and Munkvad explain that the stereotyped behaviours induced by amphetamines, which increase dopamine activity, are selective. Some specific behaviours are increased in frequency, whereas others are decreased. The authors note that in lower doses, behaviours such as sniffing, locomotion and rearing occur, and there is a decrease in grooming. At higher doses, grooming continues to decrease and eventually disappears, and both locomotion and rearing also begin to decrease as doses increase (Randrup & Munkvad, 1974). This corresponds to literature on NMDA antagonists which describes a similar pattern of stereotypy as discussed below. The antagonism of stereotypy has also been shown with both typical antipsychotics such as haloperidol and atypical antipsychotics such as clozapine, thus further supporting the glutamate hypothesis of schizophrenia (Tiedtke, et al., 1990).

It has been shown that ketamine induces stereotyped behaviours, such as circling, when administered either chronically (Leccese, Marquis, Mattia, & Moreton, 1986) or acutely (Wilson, et al., 2005). Other stereotyped behaviours frequently recorded are reverse locomotion and head weaving (Kos et al., 2006; Wilson, et al., 2005; Wilson et al., 2007). Therefore, in the present study, reverse locomotion (backing), head weaving and circling were recorded and then combined to produce one overall measure of stereotypy, as has typified previous research (Zuardi, Rodrigues, & Cunha, 1991).

1.5 Cannabinoids

Cannabinoids are a family of molecules that display pharmacological properties by acting on cannabinoid receptors. At this point in time, the two cannabinoid receptors that have been identified are CB_1 and CB_2. CB_1 receptors are normally found in the central and peripheral nervous systems, whereas CB_2 receptors are normally found in the immune system (Pertwee, 2005). An increasing body of research supports the involvement of the CB_1 receptor in the aetiology of schizophrenia, and there is new emerging evidence to suggest a role of the CB_2 receptor (Ishiguro et al., 2010). Cannabinoids can belong to one of three groups: endogenous endocannabinoids, synthetic, or phytocannabinoids. Phytocannabinoids are those extracted from the *Cannabis sativa* plant and include Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD). Cannabinoids have been found to possess therapeutic capability in a range of areas. However, due to the psychotropic effects of THC, research surrounding its therapeutic use is the subject of much contentious debate. In contrast, CBD lacks the psychotropic effects of THC, and is therefore rapidly becoming a focus of therapeutic cannabinoid research.

1.5.1 Cannabidiol

Cannabidiol (CBD) is a non-psychoactive component that makes up to 40% of the *Cannabis sativa* plant (Iuvone, Esposito, De Filippis, Scuderi, & Steardo, 2009). The substance was first isolated in 1940 by Adams and colleagues, and in 1963, Mechoulam and Shvo ascertained its structure and chemistry (Izzo, Borrelli, Capasso, Di Marzo, & Mechoulam, 2009; Scuderi et al., 2009). Although the mechanisms of action of CBD are not entirely understood (Grotenhermen, 2005), it has been shown that it has a low affinity for both CB1 and CB2 receptors (Mechoulam, Peters, Murillo-Rodriguez, & Hanus, 2007; Scuderi, et al., 2009) as well as 5-HT1A receptors (Izzo, et al., 2009).

Recent literature has described a range of therapeutic effects of CBD (Mechoulam, et al., 2007). The compound has been shown to reduce anxiety induced by THC, thereby indicating an anxiolytic effect (Koethe, et al., 2009). It has also been suggested as a drug for neurodegenerative disorders due to its antioxidant, anti-inflammatory and neuroprotective actions (Iuvone, et al., 2009). Since, CBD toxicity in humans is extremely low (Iuvone, et al., 2009), its potential for therapeutic use is considerable.

The *Cannabis sativa* plant has been linked to the development of schizophrenia in its users. However, this link may be confined to the chronic use of THC. In actual fact, CBD has been shown to attenuate the effects of THC (Emrich, et al., 1997), and to resemble the profile of current antipsychotic drugs, thus insinuating antipsychotic properties. A study by Morgan and Curran examined the levels of THC and CBD in cannabis users, and found that individuals who showed only levels of THC, presented with higher levels of positive schizophrenic-like symptoms than those with a combination of THC and CBD, supporting the opposing actions of the two constituents (Morgan & Curran, 2008). As CBD has potential

as a new and natural antipsychotic, further understanding of its effectiveness is valuable for those individuals suffering from schizophrenia who do not respond to more conventional methods of treatment.

Zuardi and colleagues (Zuardi, Shirakawa, Finkelfarb, & Karniol, 1982) first discovered the antagonistic effect of CBD during a study of its interaction with THC. The authors found that the combination of the two drugs significantly attenuated the effects induced by the THC, and that this was not due to an interaction effect (Zuardi, et al., 1982). This was further investigated in a significant study (Zuardi, et al., 1991), in which several doses of CBD were compared with haloperidol (a typical antipsychotic) in male rats. CBD was shown to have similar effects to atypical antipsychotics, namely attenuating stereotyped behaviours (such as sniffing and biting) induced by apomorphine (a non-selective dopamine agonist). It was also shown that unlike haloperidol, CBD did not induce catalepsy (a movement disorder that involves the rigidity of muscles). A subsequent development was a study in which it was demonstrated that the antipsychotic effects of CBD were similar to those of haloperidol in a 19-year old patient suffering from schizophrenia (Zuardi, Morais, Guimaraes, & Mechoulam, 1995),.

Moreira and Guimares (2005) further assessed the antipsychotic effects of CBD by testing its effectiveness in inhibiting the hyperlocomotion induced by both dopamine (using d-amphetamine) and glutamate (using ketamine) models of schizophrenia in male mice. These effects were compared to the typical antipsychotic haloperidol, and atypical antipsychotic clozapine. CBD was shown to inhibit the hyperlocomotion induced by D-amphetamine, thus further supporting the dopamine model. Hyperlocomotion induced by ketamine was also inhibited by CBD, thereby providing additional support for the

glutamatergic model of schizophrenia, and adding support to the antipsychotic properties of CBD. Although clozapine and haloperidol also both inhibited the induced hyperlocomotion, they also decreased locomotion as compared to the control group. In contrast, CBD itself did not decrease locomotion (Moreira & Guimaraes, 2005). In accordance with earlier work by Zuardi et al. (1991), CBD and clozapine did not cause catalepsy, whereas haloperidol did, again suggesting that CBD may have a similar profile to the atypical antipsychotics.

1.6 Sex Differences

As previously noted, significant sex differences have been found between men and women who develop schizophrenia. Women have been shown to have a later onset than men, and tend to present with fewer negative and cognitive symptoms (Lees, et al., 2004). Since males and females present with schizophrenia differently, it is important to include both sexes when conducting research into schizophrenia and its potential treatments.

Ketamine, as discussed earlier, has been used to induce schizophrenic-like symptoms in both healthy volunteers, and in animal models. As with many areas of research, males (both animal and human) have been predominantly used. Prior to 2006, all previous human studies on ketamine at the time had either been conducted with male volunteers exclusively (for example see Vollenweider, et al., 2000), or with both males and females but in the absence of any distinctions being made between the two sexes (for example see Umbricht, et al., 2000). Consequently, a call was made for more studies investigating possible sex differences in responsiveness amongst human volunteers (Lees, et al., 2004). The first study to investigate sex differences in ketamine's effects on humans showed that male volunteers performed worse on a memory test than females, which is in line with sex differences in schizophrenia (Morgan, Perry, Cho, Krystal, & D'Souza, 2006). In animal models, although

the majority of research has also used males (Chatterjee, et al., 2010; Garcia et al., 2008; Hayase, et al., 2006; Leite, et al., 2008; Rodvelt, et al., 2008) there is now an increasing body of research that suggests females are more sensitive to ketamine than males. For example, in an early study Winters et al. (1986) found sex differences in analgesia, and that female rats lost their righting reflex for significantly longer than males, even though they were injected with a lower dose of ketamine (80mh/kg vs. 100mg/kg). A more recent study Wilson et al. (2005) found that ketamine induced reverse locomotion and headweaving in both sexes, but that these levels were significantly higher for females than for males. Ketamine also induced turning in females, but not in males, and decreased rearing regardless of sex (Wilson, et al., 2005). These authors later reported similar results, with decreased locomotion and increased stereotypy in both sexes, and with ketamine becoming ineffective for older males but not for females (Wilson, et al., 2007).

To date, no research investigating the antipsychotic effects of CBD has considered possible sex differences. Furthermore, few major studies using CBD in research in any field have examined sex differences. With regards to antipsychotic research, all rodent studies have used males (Moreira & Guimaraes, 2005; Zuardi, et al., 1991). Zuardi et al. (2006) used a small sample with both male and females human participants, but possible sex differences were not considered, as with an investigation of CBD's effects on Parkinson's disease (Zuardi et al., 2009). Most other CBD research has also involved males exclusively. Notable examples are studies the behavioural effects of CBD (Long et al., 2010) and its effects on stress (Bitencourt, Pamplona, & Takahashi, 2008; Resstel et al., 2009). This leaves a significant gap in current CBD research generally and specifically in antipsychotic research.

2.0 Aims and Hypotheses

The present research was intended to further investigate the antipsychotic potential of CBD by investigating its effectiveness in a glutamate (ketamine) animal model of schizophrenia, taking into account possible sex differences in responsiveness to the compound. The main limitation of previous research, as already discussed, is the lack any consideration of potential sex differences in the antipsychotic effects of CBD. The current research involved both male and female rats and both a high and low dose of CBD.

As mentioned previously, it is hypothesised that the glutamate system may be involved in the aetiology of schizophrenia, and that antagonists of the NMDA glutamate receptor are able to induce positive and negative symptoms as well as cognitive symptoms. Since ketamine has a range of effects that are likely to be induced by NMDA antagonism, the current research involved the use of ketamine as a model for schizophrenia. The reason for using ketamine rather than PCP is that, since PCP is no longer used in human studies, more information about ketamine could be useful for future research (Jentsch & Roth, 1999). The acute effects of CBD were accordingly tested after ketamine administration.

Firstly, it was hypothesised that the administration of ketamine would induce schizophrenic-like behaviours in the rat. These behaviours include hyperlocomotor activity, stereotypic behaviours and learning and memory deficits, as measured in an open field and Y maze. Decreases in 'normal' behaviours such as grooming (Randrup & Munkvad, 1974) were also included. Secondly, based on findings from previous research, it was hypothesised that there would be significant sex differences in ketamine's effects. Specifically, it was hypothesised that females would show higher levels of locomotor activity. It was also hypothesised that ketamine would induce more stereotyped behaviour in the females than in

the males. This seemed likely in view of previous research describing sex differences in the same stereotyped behaviours (circling, backing and head weaving, Wilson, et al., 2005). Because of expected sex-related differences, the data from male and female rats were statistically analysed separately for effects of CBD on the action of ketamine.

It was hypothesised that CBD would alter the effects induced by the ketamine. It was proposed that CBD would modify any effects of ketamine so that the outcomes would be similar to those of the saline condition. In line with previous research, it was not expected that CBD on its own would decrease locomotion (Moreira & Guimaraes, 2005). Therefore, any changes observed between the ketamine-only and the ketamine/CBD conditions would be attributed to a combination of the two drugs. Specifically, it was hypothesised that CBD would decrease ketamine-induced hyperlocomotion (Moreira & Guimaraes, 2005) and, in line with previous observations (Zuardi et al., 1991), would attenuate ketamine-induced stereotyped behaviours. As there is no previous research on record of sex differences in responsiveness to CBD, it was not possible to predict how males and females would react.

3.0 Method

3.1 Subjects

The subjects were 24 male and 24 female PVG/C Hooded Rats, which were breed at the University of Canterbury, New Zealand. The rats were housed in groups of three of the same sex in large cages, with free access to communal food and water. The temperature-controlled environment (temperature 22 (± 2)^{O} C and humidity 48 (± 10)%) was on a 12 hr light/dark cycle. All testing occurred during the light cycle.

Cages were randomly assigned to one of four experimental groups. These were as follows: the saline only control group (saline injection followed by second saline injection); ketamine only group (ketamine injection followed by saline injection); cannabidiol low dose group (ketamine injection followed by a cannabidiol injection of 10mg/kg); and a cannabidiol high dose group (ketamine injection followed by a cannabidiol injection of 20mg/kg). All testing occurred between the ages of PND170 and PND245, during adulthood. All procedures were approved by the University of Canterbury Animal Ethics Committee (see Appendix A).

3.2 Drugs and Rationale for Doses

Ketamine was purchased from Provet NZ in a concentration of 100mg/ml and was diluted in sterile 0.9% saline to be administered via intraperitoneal injection (i.p.) in a dose of 30mg/kg. This dose was based on previous research which has predominantly used doses between 30mg/kg and 50mg/kg in rats (Becker, et al., 2003; Hayase, et al., 2006; Keilhoff, et al., 2004; Rodvelt, et al., 2008). Using a dose at the lower end of this range was decided upon because of an indication from other unpublished research within the department suggesting

that PVG/C rats may be particularly sensitive to drugs, and it has been reported that ketamine effects may be affected by strain of rat (Large, 2007).

Dr. Raphael Mechoulam (Hebrew University, Jerusalem, Israel) kindly supplied powdered cannabidiol (CBD). It was dissolved in Tween-80 and sterile 0.9% saline (Bitencourt, et al., 2008; Moreira & Guimaraes, 2005) and administered via intraperitoneal injection at a dose of either 10mg/kg or 20mg/kg. A fresh batch of the drug was made up daily before administration. These doses were based on previous research where CBD has been administered to rats in doses ranging from 1mg/kg- 60mg/kg (Moreira & Guimaraes, 2005; Resstel, et al., 2009; Zuardi, et al., 1991). As noted earlier, it appears that the rats used within the department may be particularly sensitive to drugs, thus lower doses of CBD were selected for use. All solutions were injected at a volume of 1mL/kg.

Table 1: ***Conditions and doses for male and female rats. First injection (horizontal) by second injection (vertical)***

	Ketamine 30mg/kg	Saline 1mL/kg
Saline 0.9% 1mL/kg	6 M 6 F	6 M 6 F
CBD 10mg/kg	6 M 6 F	-
CBD 20mg/kg	6 M 6 F	-

3.3 Materials

Rats underwent testing in both an open-field chamber and a Y-maze apparatus. These two pieces of equipment are explained in more detail below, followed by an explanation of procedure and assessment of behaviours that were recorded during these tests.

3.3.1 Open field

The open field apparatus comprised a 60 X 60 X 30cm box with transparent Perspex walls. The floor was black and divided into a white-lined grid of 16 numbered squares. A timer with an ear piece that produced an auditory signal every three seconds was also used to record the rats position and behaviour in the chamber.

3.3.2 Y-Maze

The wooden Y maze consisted of two 45cm long arms and a 15cm long stem, all 10cm wide and 14cm high. A clear Perspex lid covered the top of the entire Y-maze. Black and white aluminium inserts could fit into the arms of the maze, to test for responsiveness to change and short-term spatial memory (see sections 1.4.2 and 3.4.1.2). The experimenter used a PC computer and keyboard with specialised software to record the movement of each rat within the maze.

3.4 Procedure

The subjects were randomly allocated to one of 4 groups in each of which were 6 males and 6 females. One group received intraperitoneal saline (0.9%) alone followed immediately by another saline injection (as per the procedure for ketamine-treated rats described below). This served as a control for the rats that received ketamine and water, in order to provide a baseline for the changes that the ketamine alone induced. All other rats received ketamine (30mg/kg) via intraperitoneal injection, followed immediately by the relevant dose of saline or CBD (also via intraperitoneal injection).

3.4.1 Behavioural Assessment

All testing occurred within the light phase of the light/dark cycle. Rats were injected and tested on both behavioural tests (open-field and Y-maze) during the same day, and this was the only behavioural testing each animal experienced. The rats were injected in a separate testing room from where they were housed. Twenty minutes post-injection, the rat was tested in the open-field, and then in the Y maze, before being returned to its cage. The testing apparatus was cleaned with a solution of 20% Powerquat blue and 80% water after each use.

3.4.1.1 Open-Field Testing

Twenty minutes post injection, the rat was placed into the centre of the open field and locomotor activity and stereotypic behaviour were recorded for five minutes. Every three seconds, the location of the rat was recorded, as were any behaviours that rat was engaged in. The location of the rat allowed the researcher to estimate the general locomotor activity by calculating the number of changes that were recorded for that animal during the five minutes. Instances of normal behaviours, rearing and grooming, were recorded every three seconds during the five-minute trial. Faecal boluses for each rat at the end of the test were also recorded. Higher levels of defecation and grooming are an indication of increased emotionality in the rat, whereas lower levels of exploratory behaviours (such as rearing) are an indication of anxiety (Belzung, 1999).

Instances of abnormal “stereotypic” ketamine-induced behaviours were also recorded, namely circling, backing, and head weaving. Using definitions from previous research, circling was recorded when the rat rotated its body in a complete turn; backing was recorded

when the rat took two or more steps backwards; and head weaving was defined as “the rat slowly and rhythmically weaving its head from side to side” (Wilson, et al., 2007, p. 204).

3.4.1.2 Y-maze Testing

Immediately after completion of the open-field test, the rat was placed in the proximal end of the stem of a wooden Y maze. The Y-maze test measures responsiveness to change, anxiety, and short-term spatial memory (Hughes & Maginnity, 2007).

The test began with a 6-min acquisition trial in which the rat was able to move freely between the two arms of the maze. One of the arms contained a black insert, and one a white. Half or the rats were randomly allocated to a group in which the white insert was in the right arm and, for the other half, it was in the left. At the end of the acquisition trial, the rat was removed while the arm inserts were replaced with two clean black inserts meaning that, one arm remained unchanged while the other (novel) changed arm had changed from white to black. The rat was again placed in the stem of the maze, this time for a 3-min retention trial in which the first arm entered (novel or unchanged), time spent in each arm, and the number of entries to each arm were recorded by an observer making use of PC and keyboard. An estimate of locomotor activity was calculated by recording the total number of entered in to both arms. Typically, rats will enter the changed arm first, and will make more entries and spend more time in the novel/changed arm (Hughes, 2004).

4.0 Statistical Analysis

This study intended to examine the effects of both low dose and high dose administration of CBD after ketamine administration. The hypothesis was that cannabidiol would alleviate ketamine-induced effects. The statistical program *Statistica 9* was used for all analyses. Separate 4 (condition) x 2 (sex) factorial AVOVAs were performed on each measure, and post hoc Fishers PLSD tests were used for comparisons between specific groups when main effects were significant.

Males and females were analysed separately, since it was expected that there would be significant differences between sexes due to ketamine administration- shown by previous research.

5.0 Results

Throughout the results section the conditions will be referred to as follows:
S/S= saline injection followed by second saline injection (control group); K/S= ketamine injection followed by saline injection; K/10= ketamine injection followed by cannabidiol low dose injection of 10mg/kg; and K/20= ketamine injection followed by cannabidiol higher dose injection of 20mg/kg.

Since it was highly likely that the two sexes could differ significantly in the effects of ketamine (Wilson, et al., 2005; Wilson, et al., 2007), one-way ANOVAs were performed on each behavioural measure for males and females separately. Overall sex differences (for all drug groups combined) were determined by separate F tests.

5.1 Open-field Results

For the purposes of minimising the effects of high variance and thus facilitating the application of ANOVAs to the data, individual scores of 0 for defaecation and stereotypy were converted to 0.01. Also, the different examples of stereotyped behaviour (circling, head weaving and backing) were summed to produce a single stereotypy score, meaning $F(3,68)$ in Table 1 and $F(1,142)$ in Table 2.

Effects of the drug treatments on each individual open-field measure for males and female separately are described in the following subsections. Results of the ANOVAs for these treatments can be seen in Table 2. Overall sex differences for each open-field response are outlined in Table 3.

Table 2: ***Results of ANOVAs for effects of the drug treatments on males and females separately***

Male	*F* (3,20)	*P*
Rearing	87.265	<0.0001
Grooming	3.2642	<0.05
Defecation	3.3900	<0.05
Locomotion	21.993	<0.001
Centre Occupancy	2.9171	0.059
Corner Occupancy	1.2562	0.319
Stereotypy	3.50	<0.05
Female Condition	***F*** **(3,20)**	***P***
Rearing	155.3945	<0.0001
Grooming	8.1929	<0.001
Defecation*	4.0000	<0.05
Locomotion	3.0305	0.053
Centre Occupancy	4.2306	<0.05
Corner Occupancy	8.0163	<0.01
Stereotypy	5.42	<0.01

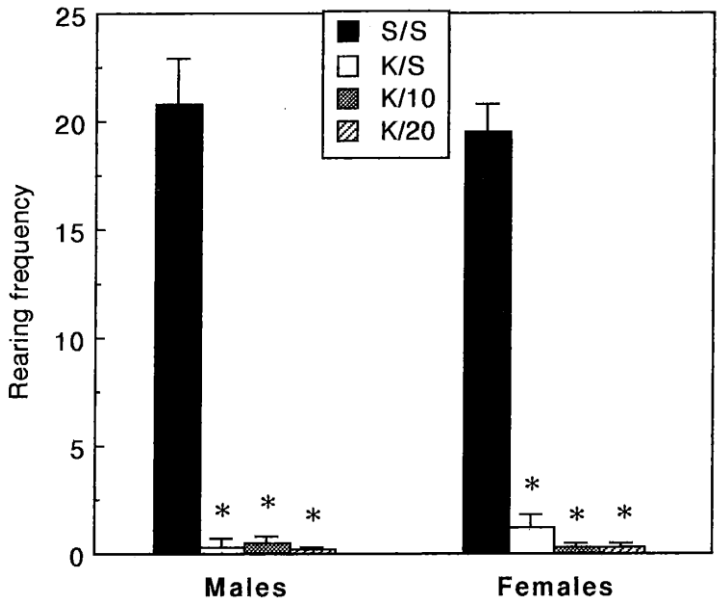
Table 3: ***Mean (± SEM) frequencies of each open-field measure for male and female rats (all drug groups combined), and results of F tests for sex differences***

Sex	Male (n=24)	Female (n=24)	*F* (1,46)	*P*
Rearing	5.46 (1.92)	5.33 (1.74)	0.0023	0.962
Grooming	1.08 (0.42)	2.46 (1.06)	1.4459	0.235
Defecation*	0.71 (0.26)	0.17 (0.10)	3.8296	0.056
Locomotion	36.04 (3.03)	42.29 (3.57)	1.7803	0.189
Centre Occupancy	33.88 (5.07)	39.75 (5.87)	0.5734	0.453
Corner Occupancy	26.63 (3.77)	20.92 (3.71)	1.1626	0.287
Stereotypy*□	3.63 (0.97)	16.75 (3.13)	16.06	<0.001

5.1.1 Rearing

Effects of the drug conditions on rearing for males and females separately are outlined in Figure 1.

Figure 1: ***Mean (± SEM) frequencies of rearing for male and female rats separately following each type of drug treatment.***



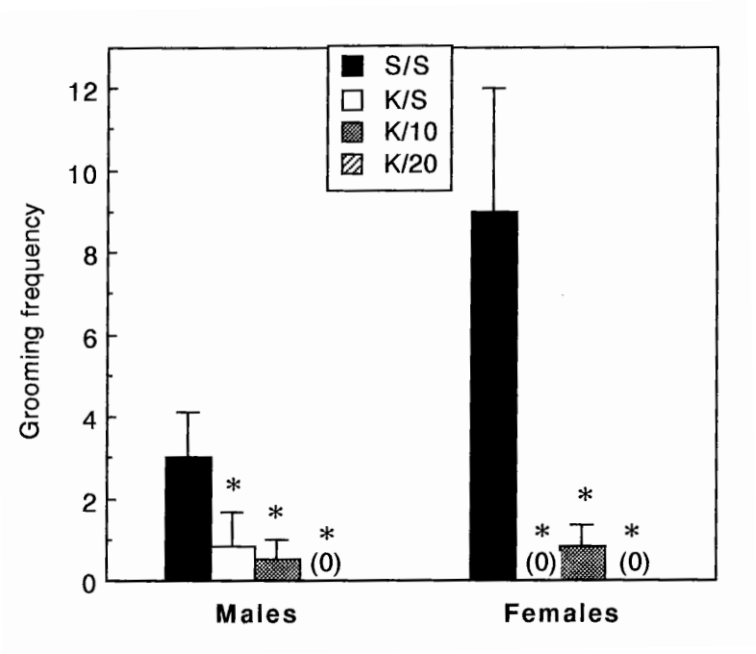
*significantly different from control (S/S) group ($P<0.05$, Fishers PLSD test).

There was a significant effect of condition on rearing with all drug groups showing significantly less rearing than the controls for both male and female rats. There were no significant overall sex differences.

5.1.2 Grooming

Effects of the drug conditions on grooming for males and females separately are outlined in Figure 2.

Figure 2: ***Mean (± SEM) frequencies of grooming for male and female rats separately following each type of drug treatment.***



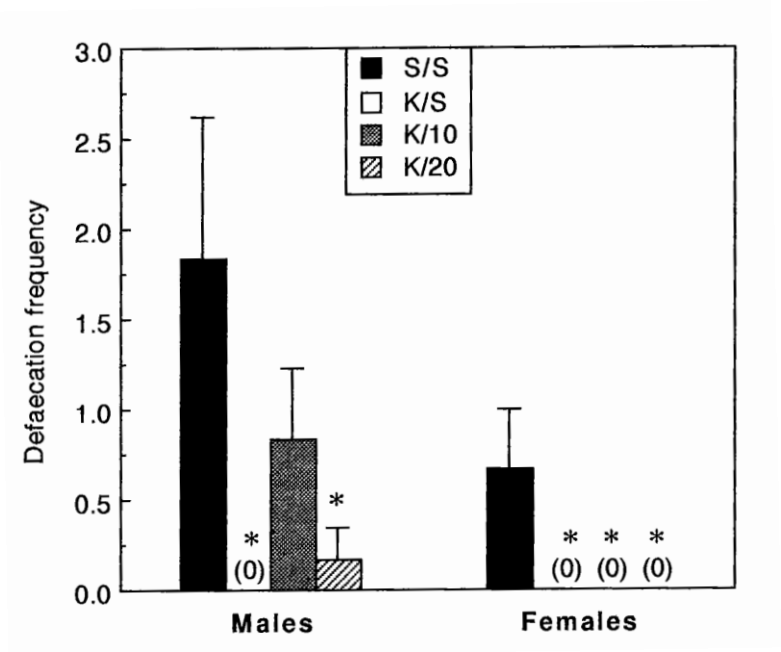
*significantly different from control (S/S) group ($P<0.05$, Fishers PLSD test).

Grooming was significantly decreased in all drug conditions compared to controls for both male and female, and there was no significant overall sex difference.

5.1.3 Defecation

Effects of the drug conditions on defecation for males and females separately are outlined in Figure 3.

Figure 3: ***Mean (± SEM) frequencies of defaecation for male and female rats separately following each type of drug treatment.***



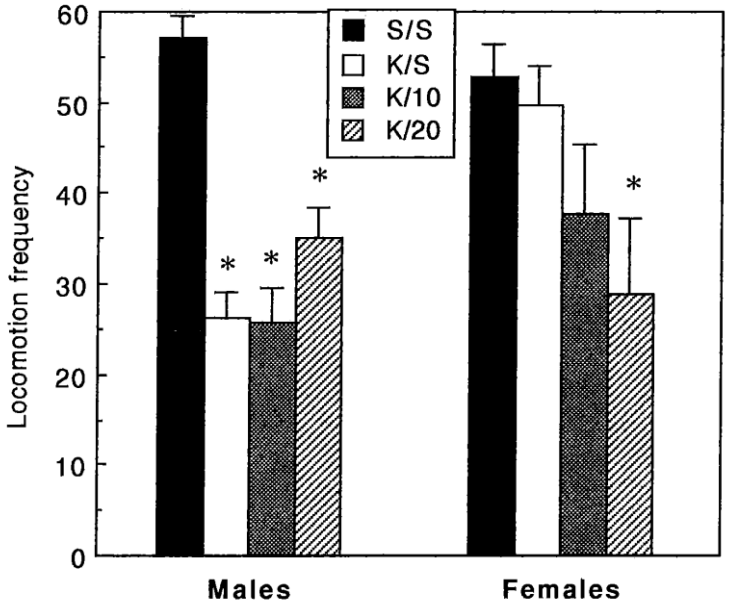
*significantly different from control (S/S) group (P<0.05, Fishers PLSD test).

There was a significant effect of condition with rats in each drug condition producing fewer faecal boluses than S/S rats for both male and female. There was no significant overall sex difference.

5.1.4 Locomotor Activity

Effects of the drug conditions on locomotor activity for males and females separately are outlined in Figure 4.

Figure 4: ***Mean (± SEM) frequencies of locomotor activity for male and female rats separately following each type of drug treatment.***



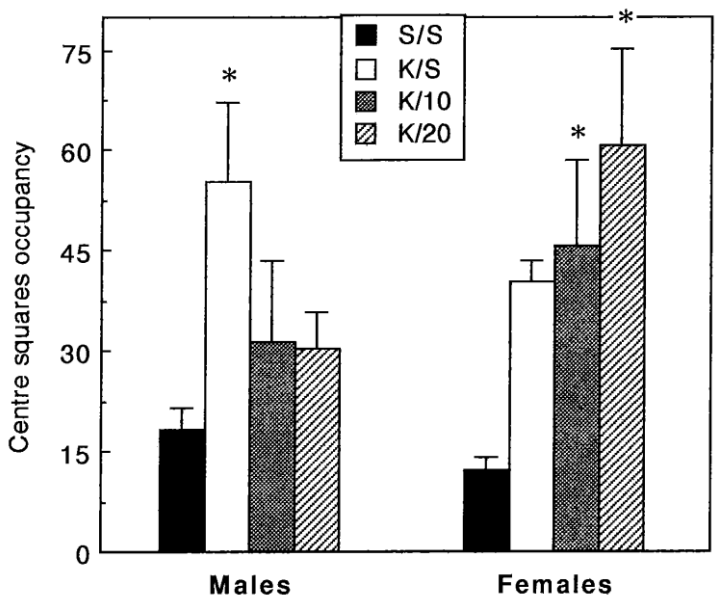
*significantly different from control (S/S) group (P<0.05, Fishers PLSD test).

For males, there was a significant effect of condition with rats in each drug condition having less locomotor activity than controls. For females, there was a significant effect of condition on locomotor activity with ambulation decreasing across each drug condition from S/S. There was no significant overall sex difference.

5.1.5 Centre Occupancy

Effects of the drug conditions on centre occupancy for males and females separately are outlined in Figure 5.

Figure 5: ***Mean (± SEM) frequencies of centre occupancy for male and female rats separately following each type of drug treatment.***



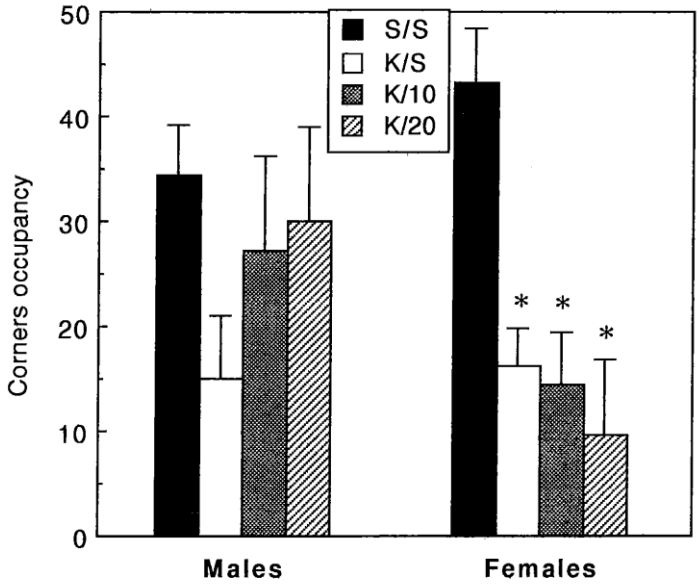
*significantly different from control (S/S) group ($P<0.05$, Fishers PLSD test).

There was significantly more centre occupancy for all drug conditions for both male and female than for S/S, but there was no significant overall sex difference.

5.1.6 Corner Occupancy

Effects of the drug conditions on corner occupancy for males and females separately are outlined in Figure 6.

Figure 6: ***Mean (± SEM) frequencies of corner occupancy for male and female rats separately following each type of drug treatment.***



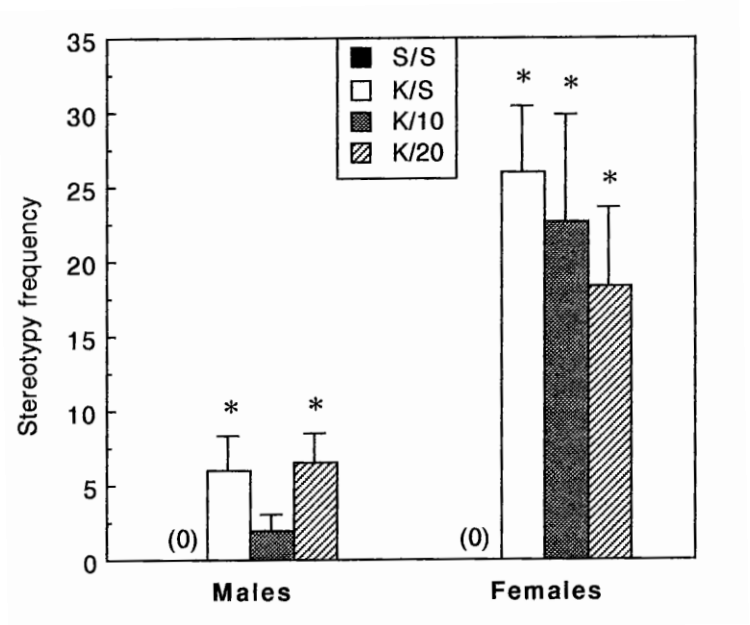
*significantly different from control (S/S) group ($P<0.05$, Fishers PLSD test).

There was significantly less corner occupancy for all drug conditions than for S/S for both male and female, but there was no significant overall sex difference.

5.1.7 Stereotypy

Effects of the drug conditions on stereotypy for males and females separately are outlined in Figure 7.

Figure 7: ***Mean (± SEM) frequencies of stereotypy for male and female rats separately following each type of drug treatment.***



*significantly different from control (S/S) group ($P<0.05$, Fishers PLSD test).

Stereotypy was significantly higher in all drug conditions than controls for both sexes, and a significant sex difference revealed that females display more stereotypy than males.

5.2 Y-Maze Results

Effects of the drug treatments on each individual Y-maze measure for males and female separately are described in the following subsections. Results of the ANOVAs for these treatments can be seen in Table 4. Overall sex differences for each Y-maze measure are outlined in Table 5.

Table 4: ***Results of ANOVAs for effects of the drug treatments on males and females separately.***

Male Condition	*F* (3,20)	*P*
Total Time in Both Arms	2.0895	0.134
% Time in Novel	3.66	<0.05
Total Entries Both Arms	7.0206	<0.01
% Entries Novel	1.8397	0.173
Female Condition	***F*** **(3,20)**	***P***
Total Time in Both Arms	1.8652	0.168
% Time in Novel	0.61	0.619
Total Entries Both Arms	0.2522	0.859
% Entries Novel	0.4439	0.724

Table 5: ***Mean (± SEM) frequencies of each Y-maze measure for male and female rats (all drug groups combined), and results of F tests for sex differences***

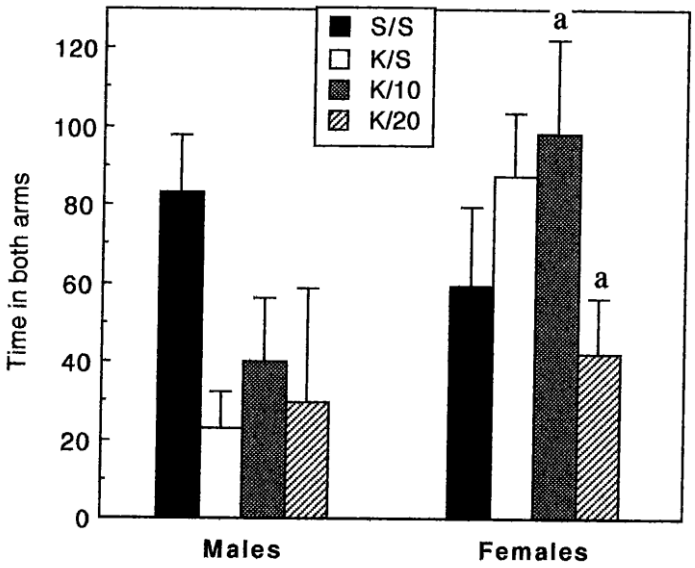
Sex	Male (n=24)	Female (n=24)	*F* (1,46)	*P*
Total Time in Both Arms	43.91 (10.08)	71.76 (9.98)	3.8542	.056
% Time in Novel	6.93 (1.68)	19.29 (4.23)	8.5100	.006
Total Entries Both Arms	1.88 (0.40)	3.50 (0.34)	9.5504	<.01
% Entries Novel	43.08 (7.35)	55.35 (5.30)	1.8336	.182

***** *F*(1,6) because the means of each group were compared against each other, not individual subject scores.

5.2.1 Total Time Spent in Both Arms

Effects of the drug conditions on the total amount of time spent in both arms for males and females separately are outlined in Figure 8.

Figure 8: ***Mean (± SEM) total amount of time spent in both arms for male and female rats separately following each type of drug treatment.***



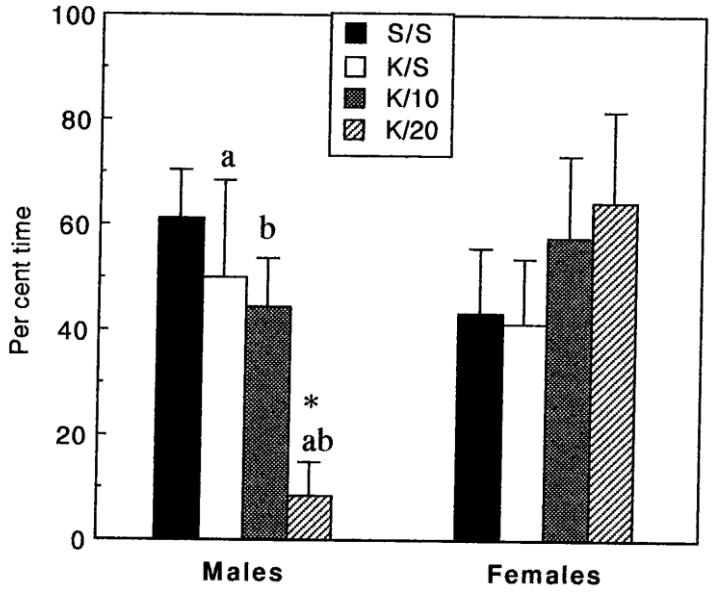
*significantly different from control (S/S) group (P<0.05, Fishers PLSD test).
The difference between the two groups with superscripts in common is significant (Fisher PLSD test, P<0.05)

There was no significant difference of condition for either sex, nor any significant interactions.

5.2.2 Percentage of Time Spent in the Novel Arm

Effects of the drug conditions on the percentage of time spent in the novel arm for males and females separately are outlined in Figure 9.

Figure 9: ***Mean (± SEM) percentage of time spent in the novel arm for male and female rats separately following each type of drug treatment.***



*significantly different from control (S/S) group (P<0.05, Fishers PLSD test).

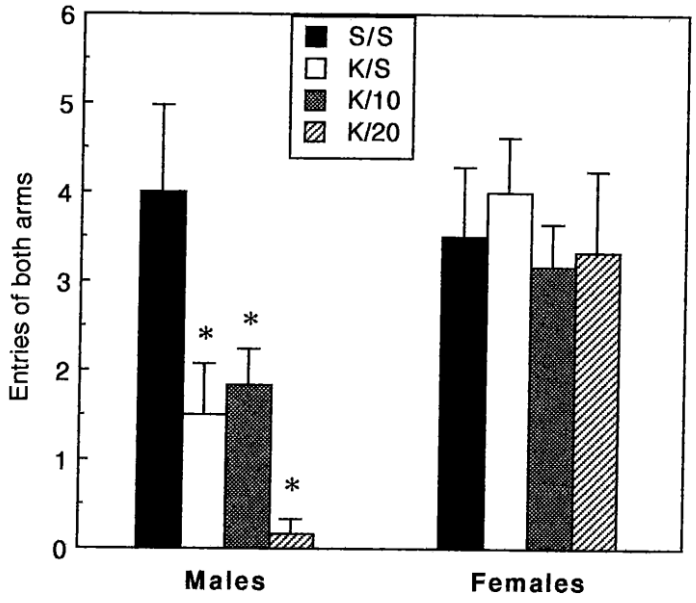
The difference between the two groups with superscripts in common is significant (Fisher PLSD test, P<0.05)

For males, there was a significant effect of condition with rats in each drug condition spending less percentage of time in the novel arm than controls. There was no significant condition effect for females, nor a significant overall sex difference.

5.2.3 Total Number of Entries in Both Arms (Locomotor Activity)

Effects of the drug conditions on the total number of entries in to both arms for males and females separately are outlined in Figure 10.

Figure 10: ***Mean (± SEM) frequencies of the total number of entries in to both arms for male and female rats separately following each type of drug treatment.***



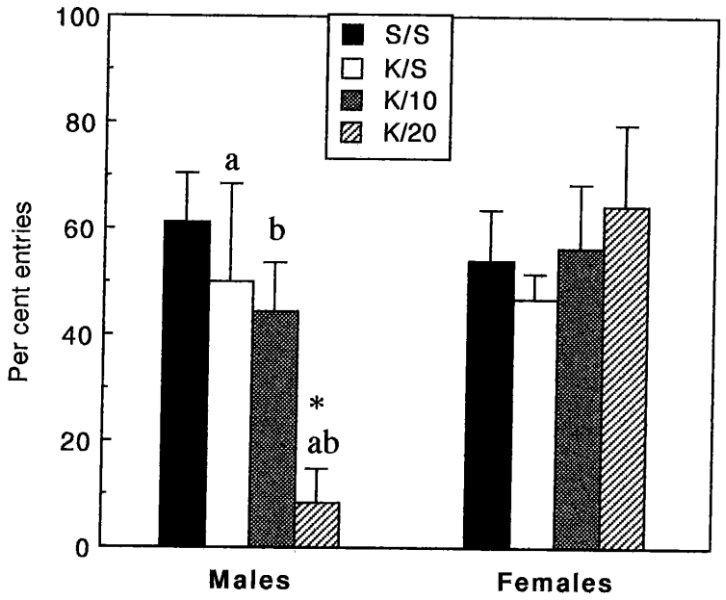
*significantly different from control (S/S) group (P<0.05, Fishers PLSD test).

General mobility scores were calculated for each condition by taking the sum of entries in to each arm. For males, there was a significant effect of condition with rats in each drug condition having lower levels of locomotor activity than controls. For females, there was no significant difference of condition, however there was a significant overall sex difference, with females having higher levels of locomotor activity than males.

5.2.4 Percentage of Entries in to Novel Arm

Effects of the drug conditions on the percentage of entries in to the novel arm for males and females separately are outlined in Figure 11.

Figure 11: ***Mean (± SEM) percentage of entries in to the novel arm for male and female rats separately following each type of drug treatment.***



*significantly different from control (S/S) group (P<0.05, Fishers PLSD test).

The difference between the two groups with superscripts in common is significant (Fisher PLSD test, P<0.05)

There was no significant difference of condition for either sex, nor any significant overall sex difference.

6.0 Discussion

6.1 General Summary

The present research aimed to further investigate the antipsychotic effects of CBD in a glutamate (ketamine) model of schizophrenia. Due to the lack of previous research investigating sex differences with respect to responsiveness to the compound, one of the primary aims of this research was to examine the possible differences. As such, both male and female rats were used. A lower and a higher dose of CBD were also examined. In this section, the findings of the study will be discussed in comparison with previous research on CBD. Strengths and limitations of the present study will also be discussed, along with implications and suggestions of further research.

Overall, the results showed that in some instances, CBD had the ability to attenuate ketamine-induced behaviours in the rat. However, due to limitations that will be discussed in this section, these results should be viewed with caution. Perhaps the most important finding was the presence of sex differences observed in the conditions involving CBD. This research highlights the lack of literature investigating sex differences in CBD responsiveness, and calls for future research to focus on this.

6.2 Ketamine-induced Effects

Based on the glutamate hypothesis of schizophrenia etiology, effects of the NMDA receptor antagonist ketamine were used as an animal model of schizophrenia. It was hypothesised that the administration of ketamine would induce a range of psychotic-like symptoms in the rat. These symptoms included an increase in locomotor activity, deficits in learning and memory, and induced stereotyped behaviours, as measured in an open field and

a Y maze. In previous research, doses ranging from 30mg/kg-100mg/kg have been used (Becker, et al., 2003; Hayase, et al., 2006; Rodvelt, et al., 2008). However, the lower dose of 30mg/kg was selected for the present research because of an as yet untested likelihood that PVG/C Hooded rats may display a heightened sensitivity to various substances, compared with some more commonly used strains.

It was hypothesised that ketamine would induce hyperlocomotion. This was based on a vast body of research in which hyperlocomotion has been used as a measure in animal models of schizophrenia (Hayase, et al., 2006; Leite, et al., 2008; Rodvelt, et al., 2008; Sams-Dodd, 1998). Contrary to the hypothesis, the administration of ketamine did not induce hyperlocomotion in either sex, and instead it decreased locomotor activity in both sexes. Ketamine decreased locomotion in males more than females, although there was no overall sex difference (see Figure 4). It is therefore possible that this lack of locomotion contributed to the lack of stereotypy observed in the male rats, as will be discussed below. These changes in locomotor activity were also observed in the Y-maze testing, with the total number of entries in to both arms being significantly reduced for males but not for females.

As noted, a substantial body of literature supports hyperlocomotion as a ketamine model of schizophrenic-like behaviour. The most likely explanation for why this research failed to come to the same conclusions as previous research is that the dose of ketamine was too high. As previously noted, it has been found that PVG/C Hooded rats appear to be particularly sensitive to some substances. Although this was taken into consideration, with a lower dose of 30mg/kg being used, this dose may have still been too high, particularly for males. This is supported by literature that suggests that higher doses of ketamine will induce

hypolocomotion, followed by hyperlocomotion (Becker, et al., 2003). If the dose was indeed too high, a longer test period may have shown an increase in locomotor activity over time.

As was expected, none of the stereotyped behaviours that were measured (circling, backing and head weaving) were present in the control groups of either sex. As hypothesised, these abnormal behaviours were successfully induced by ketamine administration in both males and females. It was also hypothesised that there would be a higher rate of stereotypy in the females than in the males. The current research supported this hypothesis, demonstrating that the females displayed significantly more stereotyped behaviours than males (see Figure 7).

At first glance this suggests that females may have been more sensitive to the effects of ketamine. This explanation is supported by previous research that has shown similar sex differences, and attributed this to possible mechanisms such as differences in hormones or metabolism, and the female oestrous cycle (Wilson, et al., 2005). However, it is important to note that when looking at these data in combination with the data on locomotor activity, it appears that males may have needed a lower dose of ketamine, suggesting that they may have been more sensitive to the substance. As has been discussed, ketamine produced a significant drop in locomotor activity for males, more so than for females. Due to a lack of voluntary movement in the males, they may not have had the opportunity to display stereotyped behaviours. Given that the females retained more movement following ketamine administration, they were probably able to display high levels of stereotyped behaviours. It is proposed that a more accurate impression of stereotypy in males would have been provided if the ketamine dose had been lower, thus retaining higher levels of locomotor activity. This would have also allowed for a more precise evaluation of sex differences in this measure.

It was also hypothesised that ketamine would induce a decrease in 'normal' behaviours. Previous research has found that grooming is significantly decreased with ketamine administration (Randrup & Munkvad, 1974). The current research produced results that were consistent with this, namely a significant decrease in grooming for both males and females but with no overall significant sex difference in this measure. Similarly, rearing was also decreased for both sexes, with no overall sex differences. Lower levels of rearing, like with other measures of exploratory behaviour, are an indication of heightened anxiety. However Wilson and colleagues (2005) noted that this decrease may be attributed to ataxia produced by ketamine, thereby causing deficits in coordination and balance which would hinder the rats' ability to rear.

It was hypothesised that ketamine would induce deficits in learning and memory, as measured in the Y maze. This apparatus has been used to test responsiveness to change in rats which is dependent on short-term spatial memory (Hughes, 2001, 2004). Typically, rats will make more entries in to the novel/changed arm and spend more time in this arm (Hughes, 2004). For the K/S conditions, the percentage of time spent in the novel arm was decreased for both sexes, but neither of these was significant. Likewise, the percentage of entries in to the novel arm was decreased for both sexes, but these were not significant. It may be that ketamine had some effects on responsiveness to change and spatial memory, but a lack of statistically significant results did not support this possibility.

Frequency of defaecation during testing in the open-field was also recorded. An increase in defaecation was used as a measure of heightened anxiety. There were normal levels of defaecation present in the S/S conditions of both sexes, and there were no instances

of defaecation in the K/S conditions for either sex. This significant reduction in defaecation is consistent with previous research which has attributed this outcome to the anxiolytic effect of the substance (Zuardi & Guimares, 1997). Centre and corner occupancy were also recorded. For both sexes, ketamine increased time spent in the centre of the apparatus, as shown by an increase in centre occupancy and a decrease in corner occupancy. This increase in centre occupancy was significant for males but not females, and the decrease in corner occupancy was significant for females but not for males. This is usually an indication of decreased anxiety, which is consistent with the results showing decreased defaecation, and the anxiolytic effect of ketamine. However, since the subjects were placed in the centre of the apparatus during testing, this may simply be a reflection of the decrease in ambulation.

6.3 Cannabidiol (CBD) effects

The main focus of this research was the antipsychotic potential of CBD in a ketamine model of schizophrenia, taking into account possible sex differences. To date, no studies have considered sex differences in responsiveness to the drug. Immediately after ketamine administration, CBD was administered in either a low (10mg/kg) or high (20mg/kg) dose, in both males and females. As with ketamine, although doses much higher than this have been used in previous research (Zuardi, et al., 1991), lower doses were adopted due to the possibility that PVG/C Hooded rats may be more sensitive to various drug effects. Based on previous research (Moreira & Guimaraes, 2005; Zuardi, et al., 1991), it was hypothesised that CBD would attenuate the ketamine-induced effects described above. It is important to note that CBD was always administered with ketamine, so possible effects of the compound can only be considered in terms of this combination.

It was hypothesised that CBD would decrease ketamine-induced hyperlocomotion, as has been previously reported (Moreira & Guimaraes, 2005). Although ketamine did not increase locomotor activity as expected, there was evidence that CBD decreased locomotion when administered post-ketamine. It has been shown that CBD on its own will not decrease locomotor activity (Moreira & Guimaraes, 2005), so although CBD was always administered in combination with ketamine, the observed changes in this study are worthy of noting. For females, both doses of CBD with ketamine decreased locomotor activity further, suggesting an additive effect of both drugs. As can be seen in Figure 4, ketamine alone did not significantly reduce locomotor activity for females, but the decrease resulting from ketamine and the high dose of ketamine was significant. If the ketamine had induced hyperlocomotion, it would be reasonable to hypothesise that CBD would have had the potential for bringing locomotor activity back down to a similar level to that of the controls. However, a further study demonstrating this is needed. For males, the CBD conditions made no difference in locomotor activity compared to the ketamine-alone condition. This highlights a sex difference in the effects that CBD may have on activity levels in the rat when co-administered with ketamine.

As noted, ketamine successfully induced stereotypy in the rat. It was hypothesised that the subsequent administration of CBD would attenuate these effects, as has been shown for other animal models of schizophrenia (Zuardi, et al., 1991). For females, the subsequent administration of the low CBD dose with ketamine decreased instances of stereotypy as compared with the ketamine only condition, however this was not significant. The high dose of CBD decreased these behaviours further, suggesting that a higher dose again may have continued this downward nonsignificant trend and may have eliminated the behaviours altogether. For males, although ketamine induced stereotypy, this was lower than for females.

It is suggested that the dose of ketamine may have been too high for males, as supported by the levels of locomotor activity exhibited by the subjects (as discussed above). For males, the low (but not the high) dose of CBD decreased ketamine-induced stereotypy, although the difference between this low dose with ketamine and ketamine on its own was not significant. Without further research, it cannot be concluded that these effects were due to the combination of CBD and ketamine, or ketamine alone. The differences in CBD effects on stereotypy were quite different for males and females, highlighting the importance of testing with both sexes.

With regard to 'normal' behaviours, CBD administration along with ketamine made little difference to grooming. For males, both doses of CBD appeared to decrease grooming further, suggesting an additive effect. For females, the low dose reinstated low levels of grooming, however the high dose did not. Again, it is important to point out sex differences in the effects of CBD administration. Similarly, ketamine almost completely diminished rearing in both males and females, and CBD administration made little difference to this measure.

It was hypothesised that ketamine could induce learning and memory deficits, and that CBD would attenuate these effects. Although ketamine did decrease the time spent in the novel arm and the percentage of entries for both males and females, these changes were not significant. Interestingly, the effects that CBD in combination with ketamine had on males and females were vastly different. As can be seen in Figure 9, for males, the low dose of CBD decreased the percentage of time spent in the novel arm, and the high dose significantly decreased this further. These results for females were the reverse with the low dose of CBD increasing the percentage of time spent in the novel arm and the high dose increasing this

again. However, none of these conditions were significant. Although this leaves contradicting results on the actions of CBD in this measure, it does highlight the sex differences of CBD under the same conditions. As would be expected, the results were remarkably similar with regards to the percentage of entries in to the novel arm. As can be seen in Figure 11, for males, the low dose of CBD with ketamine decreased the percentage of entries in to the novel arm, and this was significantly decreased further by the high dose. For females, CBD increased this measure with the low dose, and increased it further with the high dose, although none of these conditions were significant. Since no research has fully investigated sex differences in CBD, the possible reasons behind these differences are not known.

As can be seen in Figure 3, ketamine completely diminished defaecation frequency for both males and females. For males, CBD in combination with ketamine increased defaecation in both dose conditions. This did not occur for females, again highlighting sex differences. For males, centre occupancy was significantly increased by ketamine, and this was decreased by CBD administration in both dose conditions, returning to a level that was not significantly different from the S/S condition. Likewise, ketamine had decreased corner occupancy, and this was increased by both CBD conditions. For females however, both doses of CBD continued to increase centre occupancy and decrease corner occupancy, suggesting an additive effect. Males and females responded very differently to CBD in this measure, however the mechanisms causing this difference are unknown.

It is clear that there were significant sex differences in responsiveness to a combination of ketamine and CBD. This research suggests that the mechanisms of action of the combination may be different for males and females, although further research is needed to investigate this.

6.4 Strengths

This study supported the use of ketamine for inducing schizophrenic-like stereotypy in both male and female rats. Sex differences were observed, which is in line with emerging literature that suggests females are more susceptible to ketamine-induced stereotypy than males (Wilson, et al., 2005). Although ketamine did not increase locomotor activity, hypolocomotion occurred in both the open field and Y maze, thus showing consistent recording of data. Additionally, this study effectively showed the potential antipsychotic actions of CBD in showing changes in behaviour when CBD and ketamine are administered together, particularly for females. However, additional research is needed to attribute these shown changes to CBD action.

Although it was unknown if any sex differences would be present in the response to CBD, there is an increasing body of literature that suggests that sex differences are present in a range of other antipsychotic medications, and that males and females respond differently to treatment efficacy and side-effects (Smith, 2010). Although the mechanisms are not entirely known, it is thought that these discrepancies may be due to differences in hormones, metabolism, and physiology (Smith, 2010). By suggesting significant sex differences in the response to CBD in combination with ketamine, this research has not only supported these findings, but has also added new information to the field of CBD research.

6.5 Limitations

There were a number of limitations that may have compromised some of the findings reported in this study. Firstly, the dose for ketamine is thought to have been too high, particularly for the male rats. It is possible that this caused the lack of hyperlocomotion that was expected in both sexes, and also reduced stereotypy in males, therefore compromising

the ketamine model. It is suggested that this sensitivity to such a conservative dose of ketamine was due to the strain of rat. The validity of this research would have been improved if the current research had used a strain of rat that had been used previously in ketamine models, such as the Sprague-Dawley. However, this was not feasible in the university lab. A lack of previous research using a variety of strains of rat, means that, in the current study, some degree of speculation was required in selecting what doses would be appropriate for use. If the ketamine dose was in fact too high, this then provides difficulty in interpreting the results of the subsequent CBD administration. In hind sight, an initial dose-response study of ketamine in both males and females would have been valuable in determining an appropriate dose to gain the desired effects.

In addition, the current research investigated the acute effects of CBD post-ketamine administration. Improved methodology may have included measuring chronic effects of ketamine which may have mimicked the illness more closely. Previous research has used CBD as a pre-treatment to ketamine administration (Moreira & Guimaraes, 2005) or has administered a combination of two drugs at the same time (Zuardi, et al., 1991). It is unknown if any differences in results may be due to different methodologies, but it would be reasonable to assume that there may be differences in the mechanisms behind the drug effects, due to the order and/or combination of the drugs. It would have also been beneficial to have added a condition in which rats received CBD alone. This would have shown initial responsiveness to CBD and would have provided a baseline for comparison with the CBD plus ketamine conditions.

This study was conducted on a small sample size. This means that the generalisability of these results is somewhat limited, and that more significant findings may have been

revealed with a larger sample. It is always possible with the nature of such research that an experimenter bias is present while recording data. It is unlikely that this occurred, and this is demonstrated by the lack of findings which would support the hypothesis of an increase in locomotor activity in the ketamine conditions. However, it would have been ideal if the experimenter was blind to the conditions, to rule out the potential of any such bias. This was not able to be done due to the unavailability of additional personnel.

6.6 Implications

To date, no research has considered the possible sex differences of CBD in a ketamine model of schizophrenia. The current research has suggested that there appears to be sex differences in the actions of CBD in combination with ketamine. However, with the exception of the Y-maze data, these differences lack statistical significance so that more research is obviously needed. These findings have significant implications for future research using CBD both generally and specifically in antipsychotic research.

Cannabis based medications are starting to be prescribed to patients in some countries such as Canada. Some would argue that the current lack of knowledge in sex differences of these compounds means that prescribing such substances is premature. Sativex® is one example. This nasal spray is consists of a 1:1 ratio of THC and CBD (Nurmikko et al., 2007). It is primarily prescribed for pain and spasticity in multiple sclerosis, and appears to be beneficial for many patients. However, as with CBD research, research to date supporting the use of Sativex® appears to be lacking sex comparison studies. Most research has used only male participants, and those that have used both males and females have not considered sex differences. For example, a study investigating the effects of Sativex® in reducing spasticity in multiple sclerosis used both male and female participants but did not consider sex

differences (Collin, Davies, Mutiboko, & Ratcliffe, 2007). Similarly, a study on pain reported that both male and female participants could be recruited for the study, but failed to report the makeup of the actual participant pool (Nurmikko, et al., 2007). This raises the question "are possible sex differences being overlooked?" It may be that any sex differences that are present in the efficacy of this medication are minimal, but there is no way of ruling out significant differences without sufficient research. It also raises some concern over potential differences in side effects. Given that most studies have used only male participants, it is possible that there may be different side effects for females that have been overlooked. Again, this is all unknown due to a lack of relevant studies. The current research, although using only CBD and not the combination present in Sativex®, has drawn attention to a lack of research investigating sex differences in therapeutic cannabinoid research, and has indicated significant sex differences. With current research supporting CBD for a range of therapeutic uses, sex differences must be looked at more closely as we move forward in prescribing such drugs.

6.7 Future Research Directions

This study highlights several important areas for future research. Firstly, there is a major need for research that fully investigates the sex differences of CBD administration. A comprehensive sex and dose study is needed on CBD alone as it has been shown that potential sex differences are present. It is also proposed that future research using CBD in both antipsychotic studies and other fields should consider sex differences. The present study focused on three specific stereotyped behaviours, thus it is unknown what sex differences may present in other behaviours and measures of schizophrenic-like symptoms in the rat such as pre-pulse inhibition and social withdrawal. The possibilities of further research in this area are immense.

It has been clearly demonstrated that various strains of rat may show different degrees of sensitivity to certain substances (Varty & Higgins, 1994). Some differences between PVG/C Hooded and other strains have been demonstrated (Farook et al., 2001), although little research has specifically looked into drug sensitivity. However, there is an indication from other unpublished research within this laboratory suggesting that PVG/C rats may be particularly sensitive to drugs, as compared to some commonly used strains such as Sprague-Dawley and Wistars. Firstly, it would be helpful to understand the differences between these strains of rats with regards to various commonly used drugs in the animal lab. A study that examines this would be valuable in assisting future research. Such a project is necessary so that future results can be compared with other research more accurately. An alternative proposal is that future research uses a strain that is more commonly used worldwide. This would ensure more accurate results, and allow for these to be generalised more easily.

As noted, previous studies using CBD in schizophrenia research have used a range of methodologies. A study looking at possible differences of pre and post treatment would be beneficial. Overall, more research on CBD is needed. It is clear that the substance has antipsychotic potential, along with other therapeutic qualities, and due to its safety for human consumption, these avenues are worthy of further examination. With further research, a wide-scale human trial will eventually be necessary.

6.8 Conclusions

Schizophrenia is the most debilitating and costly mental illness (Combs & Muester, 2007) and affects approximately 1% of the population worldwide (Furuta & Kunugi, 2008; Julien, 2008). Many patients with this life-altering and chronic illness either do not respond

well to current treatments, or experience side effects which make medication compliance an issue. Research working towards potential alternative treatments is invaluable.

Despite its limitations, the current research has supported the potential antipsychotic effect of CBD, as shown by trends in the discussed data, particularly for reducing stereotypic behaviour in female rats. However, since CBD was always administered in combination with ketamine, a lack of statistically significant differences between the CBD conditions and ketamine alone means that more research with a larger sample size is needed, along with a CBD alone condition to be able to attribute the observed effects to CBD action. This research has also highlighted that responsiveness to CBD in combination with ketamine differs between males and females under the same conditions. More research is needed, particularly with regard to possible sex differences. Future directions have also been proposed for investigating the impact different rodent strains may have on research outcomes.

7.0 References

Amitai, N., Semenova, S., & Markou, A. (2007). Cognitive-disruptive effects of the psychotomimetic phencyclidine and attenuation by atypical antipsychotic medications in rats. *Psychopharmacology, 193,* 521-537

Andreasen, N. C. (1997). The evolving concept of schizophrenia: From Kraepelin to the present and future. *Schizophrenia Research, 28*(2), 105-109.

Asarnow, J. R., & Asarnow, R. F. (2003). Childhood-onset schizophrenia. In E. J. Mash & R. A. Barkley (Eds.), *Child Psychopathology* (2nd ed., pp. 455-485). New York: The Guilford Press.

Barlow, D. H., & Durand, V. M. (2005). *Abnormal Psychology: An Integrative Approach* (4th ed.). Belmont, CA: Wadsworth.

Becker, A., Peters, B., Schroeder, H., Mann, T., Huether, G., & Grecksch, G. (2003). Ketamine-induced changes in rat behaviour: A possible animal model of schizophrenia. *Neuro-Psychopharmacology & Biological Psychiatry 27,* 687-700.

Belzung, C. (1999). Measuring rodent exploratory behavior. In W. E. Crusio & R. T. Gerlai (Eds.), *Handbook of molecular-genetic techniques for brain and behavior research* (pp. 738-749). Amsterdam: Elsevier.

Bitencourt, R. M., Pamplona, F. A., & Takahashi, R. N. (2008). Facilitation of contextual fear memory extinction and anti-anxiogenic effects of Am404 and cannabidiol in conditioned rats. *European Neuropsychopharmacology, 18*(12), 849-859.

Breese, G. R., Knapp, D. J., & Moy, S. S. (2002). Integrative role for serotonergic and glutamatergic receptor mechanisms in the action of NMDA antagonists: Potential relationships to antipsychotic drug actions on NMDA antagonist responsiveness. *Neuroscience and Biobehavioral Reviews, 26*(4), 441-455.

Brinded, P. M. J., Simpson, A. I. F., Laidlaw, T. M., Fairley, N., & Malcolm, F. (2001). Prevalence of psychiatric disorders in New Zealand prisons: A national study. *Australian and New Zealand Journal of Psychiatry, 35*(2), 166-173.

Bubeníková-Valešová, V., Horáček, J., Vrajová, M., & Höschl, C. (2008). Models of schizophrenia in humans and animals based on inhibition of NMDA receptors. *Neuroscience and Biobehavioral Reviews, 32*, 1014-1023.

Chatterjee, M., Ganguly, S., Srivastava, M., & Palit, G. (2010). Effect of 'chronic' versus 'acute' ketamine administration and its 'withdrawal' effect on behavioural alterations in mice: Implications for experimental psychosis. *Behavioural Brain Research*. doi:10.1016/j.bbr.2010.08.001

Collin, C., Davies, P., Mutiboko, I. K., & Ratcliffe, S. (2007). Randomized controlled trial of cannabis-based medicine in spasticity caused by multiple sclerosis. *European Journal of Neurology, 14*(3), 290-296.

Combs, D. R., & Muester, K. T. (2007). Schizophrenia. In M. Hersen, S. M. Turner & D. C. Beidel (Eds.), *Adult Psychopathology and Diagnosis* (5th ed., pp. 234-285). Hoboken, New Jersey: John Wiley & Sons, Inc.

D'Souza, D. C., Abi-Saab, W. M., Madonick, S., Forselius-Bielen, K., Doersch, A., Braley, G., et al. (2005). Delta-9-Tetrahydrocannabinol Effects in Schizophrenia: Implications for Cognition, Psychosis, and Addiction. *Biological Psychiatry, 57*(6), 594-608.

D'Souza, D. C., Perry, E., MacDougall, L., Ammerman, Y., Cooper, T., Wu, Y.-t., et al. (2004). The psychotomimetic effects of intravenous Delta-9-Tetrahydrocannabinol in healthy individuals: Implications for psychosis. *Neuropsychopharmacology, 29*(8), 1558-1572.

Delay, J., & Deniker, P. (1955). Neuroleptic effects of chlorpromazine in therapeutics of neuropsychiatry. *Journal of Clinical & Experimental Psychopathology, 16*, 104-112.

Emrich, H. M., Leweke, F. M., & Schneider, U. (1997). Towards a cannabinoid hypothesis of schizophrenia: Cognitive impairments due to a dysregulation of the endogenous cannabinoid system. *Pharmacology, Biochemistry and Behavior, 56*(4), 803-807.

Farook, J. M., Zhu, Y. Z., Wang, H., Moochhala, S., Lee, L., & Wong, P. T. H. (2001). Strain differences in freezing behavior of PVG hooded and Sprague-Dawley rats: Differential cortical expression of cholecystokinin$_2$ receptors. *NeuroReport: For Rapid Communication of Neuroscience Research, 12*(12), 2717-2720.

Furuta, M., & Kunugi, J. (2008). Animal Models for Schizophrenia: A Brief Overview. In C. W. Turck (Ed.), *Biomarkers for Psychiatric Disorders* (pp. 163-184).

Fyro, B., Wode-Helgodt, B., Borg, S., & Sedvall, G. (1974). The effect of chlorpromazine on homovanillic acid levels in cerebrospinal fluid of schizophrenic patients. *Psychopharmacologia, 35*(4), 287-294.

Gao, X., Elmer, G. I., Adams-Huet, B., & Tamminga, C. A. (2009). Social memory in mice: Disruption with an NMDA antagonist and attenuation with antipsychotic drugs. *Pharmacology, Biochemistry and Behavior, 92*(2), 236-242.

Garcia, L. S., Comim, C. M., Valvassori, S. S., Reus, G. Z., Andreazza, A. C., Stertz, L., et al. (2008). Chronic administration of ketamine elicits antidepressant-like effects in rats without affecting hippocampal brain-derived neurotrophic factor protein levels. *Basic Clin Pharmacol Toxicol, 103*(6), 502-506.

Gerlach, J., Koppelhus, P., Helweg, E., & Monrad, A. (1974). Clozapine and haloperidol in a single-blind cross-over trial: Therapeutic and biochemical aspects in the treatment of schizophrenia. *Acta Psychiatrica Scandinavica, 50*(4), 410-424.

Geyer, M. A., & Moghaddam, B. (2002). Animal models relevant to schizophrenia disorders. In Kenneth L. Davis, Dennis Charney, Joseph T. Coyle & Charles Nemeroff (Eds.), *Neuropsychopharmacology: The Fifth Generation of Progress* (pp. 689-701): American College of Neuropsychopharmacology.

Grotenhermen, F. (2005). Cannabinoids. *CNS & Neurological Disorders - Drug Targets, 4*(5), 507-530.

Hansen, H. S., Petersen, G., & Hansen, H. H. (2006). Neuroprotection: Glutamate and Endocannabinoids. In E. S. Onaivi, T. Suguira & V. D. Marzo (Eds.), *Endocannabinoids: The brain and body's marijuana and beyond* (pp. 351-369). Boca Raton: Taylor & Francis.

Haracz, J. L. (1982). The dopamine hypothesis: An overview of studies with schizophrenic patients. *Schizophrenia Bulletin, 8*(3), 438-469.

Hayase, T., Yamamoto, Y., & Yamamoto, K. (2006). Behavioral effects of ketamine and toxic interactions with psychostimulants. *BMC Neuroscience, 7*(25). doi:10.1186/1471-2202-7-25

Hoffmann, H., Kupper, Z., & Kunz, B. (2000). Hopelessness and its impact on rehabilitation outcome in schizophrenia -an exploratory study. *Schizophrenia Research, 43*(2-3), 147-158.

Hughes, R. N. (2001). Responsiveness to brightness change in hooded rats: Effects of sex and procedure. *Behavioural Processes, 55*(3), 143-155.

Hughes, R. N. (2004). Responsiveness to brightness change in male and female rats following treatment with the partial agonist of the N-methyl-D-aspartate receptor, D-cycloserine. *Behavioural Brain Research, 152*(2), 199-207.

Hughes, R. N., & Maginnity, M. E. (2007). Cues used by male and female hooded rats for locating a brightness change. *Behavioural Processes, 74*, 79-87.

Ishiguro, H., Horiuchi, Y., Ishikawa, M., Koga, M., Imai, K., Suzuki, Y., et al. (2010). Brain cannabinoid CB2 receptor in schizophrenia. *Biological Psychiatry, 67*(10), 974-982.

Itokawa, M., Arinami, T., & Toru, M. (2010). Advanced research on dopamine signaling to develop drugs for the treatment of mental disorders: Ser311Cys polymorphisms of the dopamine D2-receptor gene and schizophrenia. *Journal of Pharmacological Sciences, 114*(1), 1-5.

Iuvone, T., Esposito, G., De Filippis, D., Scuderi, C., & Steardo, L. (2009). Cannabidiol: A promising drug for neurodegenerative disorders? *CNS Neuroscience & Therapeutics, 15*(1), 65-75.

Izzo, A. A., Borrelli, F., Capasso, R., Di Marzo, V., & Mechoulam, R. (2009). Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends in Pharmacological Sciences, 30*(10), 515-527.

Jentsch, J. D., & Roth, R. H. (1999). The neuropsychopharmacology of phencyclidine: From NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology, 20*(3), 201-225.

Julien, R. M. (2008). *A Primer of Drug Action: A comprehensive guide to the actions, uses, and side effects of psychoactive drugs* (11 ed.). New York: Worth Publishers.

Kake, T. R., Arnold, R., & Ellis, P. (2008). Estimating the prevalence of schizophrenia amongst New Zealand māori: A capture-recapture approach. *Australian and New Zealand Journal of Psychiatry, 42*(11), 941-949.

Kapur, S., & Remington, G. (2001). Atypical antipsychotics: New directions and new challenges in the treatment of schizophrenia. *Annual Review of Medicine, 52*, 503-517.

Keilhoff, G., Bernstein, H.-G., Becker, A., Grecksch, G., & Wolf, G. (2004). Increased neurogenesis in a rat ketamine model of schizophrenia. *Biological Psychiatry, 56*(5), 317-322.

Keltner, N. L., & Johnson, V. (2002). Aripiprazole: a third generation of antipsychotics begins? *Perspectives in Psychiatric Care, 38*(4), 157-159.

Kim, J. S., Kornhuber, H. H., Schmid-Burgk, W., & Holzmuller, B. (1980). Low cerebrospinal fluid glutamate in schizophrenic patients and a new hypothesis on schizophrenia. *Neuroscience Letters, 20*(3), 379-382.

Koethe, D., Hoyer, C., & Leweke, F. M. (2009). The endocannabinoid system as a target for modelling psychosis. *Psychopharmacology, 206*(4), 551-561.

Kölfalvi, A., & Fritzsche, M. (2008). The endocannabinoid system is a major player in schizophrenia. *Cannabinoids and the brain* (pp. 485-528). New York, NY US: Springer Science + Business Media.

Kos, T., Popik, P., Pietraszek, M., Schäfer, D., Danysz, W., Dravolina, O., et al. (2006). Effect of 5-HT_3 receptor antagonist MDL 72222 on behaviors induced by ketamine in rats and mice. *European Neuropsychopharmacology, 16*(4), 297-310.

Lahti, A. C., Weiler, M. A., Michaelidis, T., Parwani, A., & Tamminga, C. A. (2001). Effects of ketamine in normal and schizophrenic volunteers. *Neuropsychopharmacology, 25*(4), 455-467.

Large, C. H. (2007). Do NMDA receptor antagonist models of schizophrenia predict the clinical efficacy of antipsychotic drugs? *Journal of Psychopharmacology, 21*(3), 283-301.

Leccese, A. P., Marquis, K. L., Mattia, A., & Moreton, J. E. (1986). The anticonvulsant and behavioral effects of phencyclidine and ketamine following chronic treatment in rats. *Behavioural Brain Research, 22*(3), 257-264.

Lees, J., Hallak, J. E. C., Deakin, J. F. W., & Dursun, S. M. (2004). Gender differences and the effects of ketamine in healthy volunteers. *Journal of Psychopharmacology, 18*(3), 337-339.

Leifker, F. R., Bowie, C. R., & Harvey, P. D. (2009). Determinants of everyday outcomes in schizophrenia: The influences of cognitive impairment, functional capacity, and symptoms. *Schizophrenia Research, 115*(1), 82-87.

Leite, J. V., Guimaraes, F. S., & Moreira, F. A. (2008). Aripiprazole, an atypical antipsychotic, prevents the motor hyperactivity induced by psychotomimetics and psychostimulants in mice. *European Journal of Pharmacology, 578*, 222-227.

Long, L. E., Chesworth, R., Huang, X.-F., McGregor, I. S., Arnold, J. C., & Karl, T. (2010). A behavioural comparison of acute and chronic Δ^9-tetrahydrocannabinol and cannabidiol in C57BL/6JArc mice. *International Journal of Neuropsychopharmacology, 13*(7), 861-876.

Mailman, R. B., & Murthy, V. (2010). Third generation antipsychotic drugs: Partial agonism or receptor functional selectivity? *Current Pharmaceutical Design, 16*, 448-501.

Marcotte, E. R., Pearson, D. M., & Srivastava, L. K. (2001). Animal models of schizophrenia: a critical review. *Journal of Psychiatry & Neuroscience, 26*(5), 395-410.

Matthysse, S. (1974). Dopamine and the pharmacology of schizophrenia: The state of the evidence. *Journal of Psychiatric Research, 11*, 107-113.

Mechoulam, R., Peters, M., Murillo-Rodriguez, E., & Hanus, L. O. (2007). Cannabidiol--recent advances. *Chemistry and Biodiversity, 4*(8), 1678-1692.

Meltzer, H. Y. (1992). The importance of serotonin-dopamine interactions in the action of clozapine. *British Journal of Psychiatry, 160*(17), 22-29.

Moncrieff, J. (2009). A critique of the dopamine hypothesis of schizophrenia and psychosis. *Harvard Review of Psychiatry, 17*(3), 214-225.

Moreira, F., A., & Guimaraes, F., S. (2005). Cannabidiol inhibits the hyperlocomotion induced by psychotomimetic drugs in mice. *European Journal of Pharmacology, 512*, 199-205. doi:10.1016/j.ejphar.2005.02.040

Morgan, C. J. A., & Curran, H. V. (2008). Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis. *British Journal of Psychiatry, 192*(4), 306-307.

Morgan, C. J. A., Perry, E. B., Cho, H.-S., Krystal, J. H., & D'Souza, D. C. (2006). Greater vulnerability to the amnestic effects of ketamine in males. *Psychopharmacology, 187*(4), 405-414.

Morrens, M., Hulstijn, W., Lewi, P. J., De Hert, M., & Sabbe, B. G. C. (2006). Stereotypy in schizophrenia. *Schizophrenia Research, 84*(2-3), 397-404.

Muller-Vahl, K. R., & Emrich, H. M. (2008). Cannabis and schizophrenia: towards a cannabinoid hypothesis of schizophrenia. *Expert Review of Neurotherapeutics, 8*(7), 1037-1048.

Nieratschker, V., Nöthen, M. M., & Rietschel, M. (2010). New genetic findings in schizophrenia: Is there still room for the dopamine hypothesis of schizophrenia? *Frontiers in Behavioral Neuroscience, 4*. doi:10.3389/fnbeh.2010.00023

Nurmikko, T. J., Serpell, M. G., Hoggart, B., Toomey, P. J., Morlion, B. J., & Haines, D. (2007). Sativex successfully treats neuropathic pain characterised by allodynia: A randomised, double-blind, placebo-controlled clinical trial. *Pain, 133,* 210-220. doi:10.1016/j.pain.2007.08.028

Oakley Browne, M. A., Wells, J. E., & Scotts, K. M. (Eds.). (2006). *Te Rau Hinengaro: The New Zealand Mental Health Survey*. Wellington: Ministry of Health.

Parolaro, D., Realini, N., Vigano, D., Guidali, C., & Rubino, T. (2010). The endocannabinoid system and psychiatric disorders. *Experimental Neurology, 224*(1), 3-14.

Pertwee, R. G. (2005). Pharmacological actions of cannabinoids. *Handbook of Experimental Pharmacology*(168), 1-51.

Peterson, D., Pere, L., Sheehan, N., & Surgenor, G. (2007). Experiences of mental health discrimination in New Zealand. *Health & Social Care in the Community, 15*(1), 18-25.

Randrup, A., & Munkvad, I. (1974). Pharmacology and physiology of stereotyped behavior. *Journal of Psychiatric Research, 11*, 1-10.

Resstel, L. B., Tavares, R. F., Lisboa, S. F., Joca, S. R., Correa, F. M., & Guimaraes, F. S. (2009). 5-HT1A receptors are involved in the cannabidiol-induced attenuation of behavioural and cardiovascular responses to acute restraint stress in rats. *British Journal of Pharmacology, 156*(1), 181-188.

Rodvelt, K. R., Kracke, G. R., Schachtman, T. R., & Miller, D. K. (2008). Ketamine induces hyperactivity in rats and hypersensitivity to nicotine in rat striatal slices. *Pharmacology, Biochemistry and Behavior, 91*(1), 71-76.

Rollins, A. L., Bond, G. R., Lysaker, P. H., McGrew, J. H., & Salyers, M. P. (2010). Coping with positive and negative symptoms of schizophrenia. *American Journal of Psychiatric Rehabilitation, 13*(3), 208-223.

Rosenbloom, M. (2002). Chlorpromazine and the psychopharmacologic revolution. *JAMA, 287*(14), 1860-1861.

Sams-Dodd, F. (1998). Effects of dopamine agonists and antagonists on PCP-induced stereotyped behaviour and social isolation in the rat social interaction test. *Psychopharmacology, 135*(2), 182-193.

Sartorius, N., Shapiro, R., & Jablensky, A. (1974). The international pilot study of schizophrenia. *Schizophrenia Bulletin, 1*(11), 21-34.

Scuderi, C., Filippis, D. D., Iuvone, T., Blasio, A., Steardo, A., & Esposito, G. (2009). Cannabidiol in Medicine: A Review of its Therapeutic Potential in CNS Disorders *Phytotherapy Research, 23*(5), 597 - 602.

Seeman, P. (2009). Glutamate and dopamine components in schizophrenia. *Journal of Psychiatry & Neuroscience*, *34*(2), 143-149.

Smith, S. (2010). Gender differences in antipsychotic prescribing. *International Review of Psychiatry, 22*(5), 472-484.

Stahl, S. M. (2007). Beyond the dopamine hypothesis to the NMDA glutamate receptor hypofunction hypothesis of schizophrenia. *CNS Spectrums, 12*(4), 265-268.

Stevens, J., Wilson, K., & Foote, W. (1974). GABA blockade, dopamine and schizophrenia: Experimental studies in the cat. *Psychopharmacologia, 39*(2), 105-119.

Stone, J. M. (2009). Imaging the glutamate system in humans: Relevance to drug discovery for schizophrenia. *Current Pharmaceutical Design, 15*, 2594-2602.

Stone, J. M., Morrison, P. D., & Pilowsky, L. S. (2007). Glutamate and dopamine dysregulation in schizophrenia--A synthesis and selective review. *Journal of Psychopharmacology, 21*(4), 440-452.

Tiedtke, P. I., Bischoff, C., & Schmidt, W. J. (1990). MK-801-induced stereotypy and its antagonism by neuroleptic drugs. *Journal of Neural Transmission General Section, 81*(3), 173-182.

Torrey, E. F., Stieber, J., Ezekiel, J., Wolfe, S. M., Sharfstein, J., Nobel, J. H., et al. (1992). *Criminalizing the seriously mentally ill: The abuse of jails as mental hospitals*. Washington, DC: Public Citizen's Health Research Group.

Traverso, L. M., Ruiz, G., Camino, G., & De la Casa, L. G. (2008). Ketamine blocks the formation of a gustatory memory trace in rats. *Pharmacology, Biochemistry and Behavior, 90*, 305-311.

Umbricht, D., Schmid, L., Vollenweider, F. X., Hell, D., Javitt, D. C., & Koller, R. (2000). Ketamine-induced deficits in auditory and visual context-dependent processing in healthy volunteers: Implications for models of cognitive deficits in schizophrenia. *Archives of General Psychiatry, 57*(12), 1139-1147.

Varty, G. B., & Higgins, G. A. (1994). Differences between three rat strains in sensitivity to prepulse inhibition of an acoustic startle response: Influence of apomorphine and phencyclidine pretreatment. *Journal of Psychopharmacology, 8*(3), 148-156.

Vollenweider, F. X., Vontobel, P., Øye, I., Hell, D., & Leenders, K. L. (2000). Effects of (S)-ketamine on striatal dopamine: A [^{11}C]raclopride PET study of a model psychosis in humans. *Journal of Psychiatric Research, 34*(1), 35-43.

Wheeler, A., Humberstone, V., & Robinson, E. (2008). Ethnic comparisons of antipsychotic use in schizophrenia. *Australian and New Zealand Journal of Psychiatry, 42*(10), 863-873.

Wilson, C., Cone, K., Kercher, M., Hibbitts, J., Fischer, J., Van Lake, A., et al. (2005). Naloxone increases ketamine-induced hyperactivity in the open field in female rats. *Pharmacology, Biochemistry and Behavior, 81*(3), 530-534.

Wilson, C., Kercher, M., Quinn, B., Murphy, A., Fiegel, C., & McLaurin, A. (2007). Effects of age and sex on ketamine-induced hyperactivity in rats. *Physiology & Behavior, 91*(2-3), 202-207.

Winters, W. D., Hance, A. J., Cadd, G. C., & Lakin, M. L. (1986). Seasonal and sex influences on ketamine-induced analgesia and catalepsy in the rat; a possible role for melatonin. *Neuropharmacology, 25*(10), 1095-1101.

Zuardi, A. W., Crippa, J. A. S., Hallak, J. E. C., Moreira, F. A., & Guimaraes, F. S. (2006). Cannabidiol, a Cannabis sativa constituent, as an antipsychotic drug. *Brazilian Journal of Medical and Biological Research, 39*(4), 421-429.

Zuardi, A. W., Crippa, J. A. S., Hallak, J. E. C., Pinto, J. P., Chagas, M. H. N., Rodrigues, G. G. R., et al. (2009). Cannabidiol for the treatment of psychosis in Parkinson's disease. *Journal of Psychopharmacology, 23*(8), 979-983.

Zuardi, A. W., & Guimares, F. S. (1997). Cannabidiol as an anxiolytic and antipsychotic. In M. L. Mathre (Ed.), *Cannabis in Medical Practice: A legal, historical and pharmacological overview of the therapeutic use of marijuana* (pp. 133-141). North Carolina: McFarland and Company.

Zuardi, A. W., Morais, S. L., Guimaraes, F. S., & Mechoulam, R. (1995). Antipsychotic effect of cannabidiol. *Journal of Clinical Psychiatry, 56*(10), 485-486.

Zuardi, A. W., Rodrigues, J. A., & Cunha, J. M. (1991). Effects of cannabidiol in animal models predictive of antipsychotic activity. *Psychopharmacology, 104*(2), 260-264.

Zuardi, A. W., Shirakawa, I., Finkelfarb, E., & Karniol, I. G. (1982). Action of on the anxiety and other effects produced by delta 9-THC in normal subjects. *Psychopharmacology, 76*(6), 245-250.

Appendix A

Animal Ethics Committee
Secretary
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UNIVERSITY OF CANTERBURY
Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

AEC Ref: 2009/24R

6 November 2009

Michelle Collins
Department of Psychology
UNIVERSITY OF CANTERBURY

Dear Michelle

I am pleased to inform you that the Animal Ethics Committee (AEC) has approved your application entitled: "Comparing the effectiveness of Cannabidiol to Olanzapine in reducing ketamine-induced schizophrenia-like behaviour in the rat".

Approval has been granted:

(a) for the use of 74 Rats
(b) for your research project to be undertaken from 7 October 2009 to 1 March 2011. 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 MAF 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 MAF Animal Manipulation Statistical form 30 days after the completion of this project, or once every three years, which ever 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

Associate Professor Jim Briskie
Chair
Animal Ethics Committee

cc Animal Ethics Committee