

CHAPTER ONE

Cannabis: a review of use, abuse and dependence

1.1. A brief history of cannabis use

"Its seed is said to make the genitals impotent. The juice from it drives out of the ears the worms and any other creature that has entered them, but at the cost of a headache; so potent is its nature that when poured into water it is said to make it coagulate. And so, drunk in its water, it regulates the bowels of beasts of burden. The root boiled in water eases cramped joints, gout too and similar violent pains. It is applied to raw burns, but is often changed before it gets dry"

Pliny the Elder, ancient Roman historian (died approximately 79 A.D.)

Cannabis (otherwise known as marijuana, marihuana, weed, kiff, bhang, ganja, skunk, dope, mull, pot, hemp) is a plant that is grown and used throughout the world in the common forms of *Cannabis sativa* or *Cannabis indica*. As Mechoulam (1986) has described, cannabis has been used for thousands of years for medicinal, religious and hedonistic purposes. In addition, cannabis or hemp has been used to make textiles, rope, and even food. Therefore it is not surprising that in current times cannabis is alternately viewed as a recreational drug, a drug of abuse, and a substance which may have significant therapeutic value.

Cannabis has had a long and colourful history. References to it have been found in the ancient writings of Egypt, Assyria, Greece, Rome and China (Mechoulam, 1986). Cannabis is also frequently referred to in medieval literature of Arab societies and has been used in most Arab countries as a recreational drug for the last millennium (Mechoulam, 1986). The use of cannabis probably originated in central Asia or western China (Schultes, 1969). The Scythians, who first resided near the Caucasus Mountains in southern Russia, may have been the first to use cannabis as a narcotic and as a fibre for making clothes (Godley, 1995; Herodotus, 1972; How & Wells, 1989). It is

widely accepted that cannabis found its way to Europe on the backs of these ancient nomadic horseriders, who drifted east and west of central Asia, influencing many ancient civilizations, including the ancient Greeks (Merlin, 1972). In 500 B.C. the Greek historian Herodotus described the Scythian method of bathing which has been associated with the burning of cannabis for intoxication (Artamonov, 1965; How & Wells, 1989). The bath's aromatic vapors were purported to put the ancient Greek baths to shame:

"...it begins to smoke, giving off a vapour unsurpassed by any vapour-bath one could find in Greece. The Scythians enjoy it so much they howl with pleasure" (Herodotus, 1972).

In support of this account, archaeologists have excavated Scythian tombs in central Asia, dated between 500 and 300 B.C., and have found the makings of cone-shaped tents that contained dishes in which hemp seeds were found (most likely the remnants of dried flowering tops of cannabis) (Artamonov, 1965).

Cannabis has been used for its alleged healing properties for millennia. In ancient times cannabis was also known as a "holy or spiritual plant" or as a "sacred therapy" (Mechoulam, 1986; Shultes & Hofmann, 1992). The first documented case of its use dates back to 2800 B.C., when it was listed in the Emperor Shen Nung's (regarded as the father of Chinese medicine) pharmacopoeia. Therapeutic indications of cannabis are mentioned in the texts of the Indian Hindus, Assyrians, Greeks and Romans (Mechoulam, 1986). These texts reported cannabis to treat a vast array of different health problems, such as arthritis, depression, amenorrhea, inflammation, pain, lack of appetite and asthma.

Cannabis was used in ancient religious rites by Tantric Buddhists in Tibet and by followers of the Hindu religion in India. The Tantric Buddhists consumed cannabis to enhance meditation and heighten awareness, whereas certain Hindus consumed cannabis to increase longevity and to commune with the divine spirit. Interestingly, Hindu legend holds that Shiva, the supreme Godhead of many sects, was given the title, "The Lord of Bhang", because the cannabis plant was his favourite food (Shultes & Hofmann, 1992). The ancient Hindus thought the medicinal benefits of cannabis were

explained by pleasing the Gods such as Shiva. Ancient Hindu texts attribute the onset of fever with the hot breath of the Gods who were angered by the afflicted person's behaviour. Using cannabis in religious rites appeased the Gods and hence reduced the fever. Recent scientific evidence, of course, provides an alternative explanation, where the central nervous system effects of cannabis produce hypothermia (Mechoulam, 1986).

The remains of cannabis were recently found in a 1600 year old tomb in Israel containing the corpse of an adolescent woman who died while giving birth (Zias et al., 1993). Cannabis was probably used in this instance to increase the force of uterine contraction and to reduce labor pains. However, this is a contentious explanation (Pioreschi & Babin, 1993; Zias et al., 1993). Even the great Galen (129-200 A.D.), who first verified that blood travelled through veins and arteries, utilised cannabis for its therapeutic properties and mood enhancement (Merlin, 1972). In addition to Galen, other medical writers of that era such as Dioscorides (died 90 A.D.) and Pliny the Elder (died 79 A.D.) assured that cannabis use remained for centuries, through and after the Middle Ages, because the therapeutic uses of cannabis were included in their highly influential pharmacopoeias (Mechoulam, 1986). In more recent times, Queen Elizabeth I allegedly used cannabis to alleviate menstrual pain and cramping (Science and Technology Committee, 1998).

The preceding examples provide a stark contrast to the stance of governments and authorities in the 20th century who have condemned the therapeutic use of cannabis. For example, in 1937 the uses of cannabis for medicinal and recreational purposes were effectively taxed out of existence in the United States of America (U.S.A.) by the Marijuana Tax Act (Grinspoon & Bakalar, 1997). This evolved as a result of a campaign organised by the Federal Bureau of Narcotics that wished to discourage recreational use of cannabis. A contributing factor to the success of this campaign resulted from public hysteria in response to the 1936 film "Reefer Madness" which portrayed cannabis as a highly addictive drug that caused mental disorder and violence (Annas, 1997; Grinspoon & Bakalar, 1997). Finally, the Nixon Administration moved

cannabis to being a Schedule 1 drug in 1970, viewing cannabis as a highly addictive drug with no medicinal value.

1.2. Current use of cannabis

"The cannabinoids are an overlooked group of therapeutic drugs. For over a decade there have been anecdotal and clinical reports on the usefulness of cannabis preparations in treating conditions like nausea, glaucoma, and multiple sclerosis. (...) As with other medical challenges, disciplined search for active therapeutic ingredients that address health problems which are currently not well managed is now the way forward"

British Medical Journal, 8 January 2000.

Cannabis is primarily used by today's society as a recreational drug. An important consideration when evaluating the safety of cannabis use is whether cannabis has abuse liability. Historically, this argument has been polarised by those who think cannabis is an extremely dangerous drug that is highly addictive and those who view it as a benign drug which is not addictive. As with most polarised arguments, the real state of affairs probably lies somewhere between the extremes (Hall, 1995; Hall & Solowij, 1997, 1998).

The assertion that cannabis has high abuse liability is not a trivial issue since cannabis is the most widely used illicit drug in the world (Hall & Solowij, 1998). Estimates in Western countries, such as Australia, suggest that approximately 40% of people aged 14 years or older have tried cannabis (AIHW, 1998). When looking at recent use, close to 18 % of people consumed cannabis in the year prior to the survey (AIHW, 1998). Approximately 20-30% of those who have tried cannabis become weekly users and 10% of those who have ever used cannabis become daily users (Hall & Solowij, 1998). In the U.S.A. this translates into approximately 5.5 million people who use cannabis on a weekly basis (NIDA, 1991). When only young age groups are considered, particularly the 20-24 year age group, the percentages for cannabis use are doubled (Donnelly & Hall, 1994).

In light of the widespread use of cannabis, and the fact that significant numbers use it on a daily to weekly basis, further research is needed to understand its possible habit-forming nature. This is particularly important as chronic cannabis use may have

serious health and psychological consequences. While it is uncertain what these consequences might be, the following appear the most probable: chronic bronchitis, cognitive impairment while intoxicated, cannabis dependence, and an increased risk of cancers of the mouth, oesophagus or lung which may be specifically associated with the smoking of cannabis (Hall & Solowij, 1998). In addition, epidemiological evidence alleges that cannabis use may precipitate schizophrenia, or alternatively, cannabis use may be a form of "self-medication" which helps to alleviate the symptoms of schizophrenia (Hall & Degenhardt, 2000). A recent study demonstrates that schizophrenic patients have higher levels of endogenous cannabinoids (or cannabis-like substances) in their cerebrospinal fluid than controls (Leweke, Giuffrida, Wurster, Emrich, & Piomelli, 1999). This indicates that the endogenous cannabinoid system may play some role in the pathogenesis of psychosis. However, the relationship between cannabis use and schizophrenia is uncertain and again more research is needed in this area (Hall & Degenhardt, 2000).

Apart from current concern that chronic cannabis use may have serious health and psychological consequences, anecdotal evidence describes its therapeutic efficacy in easing the suffering of patients with cancer, acquired immunodeficiency syndrome (AIDS) and multiple sclerosis. Possibly as a result, there has been a resurgent interest in the medicinal potential of cannabis and the cannabinoids (Ashton, 1999). This push, however, has been dogged by the current political climate, which views cannabis as a dangerous drug. In the U.S.A. cannabis is viewed as a highly addictive substance that may predispose users to harder drugs of abuse, such as cocaine or heroin. This view is more popularly known as the so-called "gateway hypothesis". For example, the Clinton Administration in 1996 vigorously opposed new state laws that allowed the medical use of marijuana citing the "gateway hypothesis" as one of the major reasons to justify their stance (Annas, 1997).

Many countries hold similar positions however some now acknowledge the therapeutic potential of cannabis and soon patients with specific illnesses may be allowed to use it. For instance, the House of Lords recently published a report which

recommended to the British government that cannabis be legalised for medical use only (Science and Technology Committee, 1998). This is based on claims that cannabinoid derivatives offer promising therapies for many conditions, including multiple sclerosis, brain tumors, phantom-limb pain, AIDS, treatment of mild pain, asthma, chemotherapy-induced vomiting and wasting, and glaucoma (Ashton, 1999). Recent preclinical studies have provided support for some of these claims. Cannabinoids have been found to reduce tremor and spasticity in an animal model of multiple sclerosis (Baker et al., 2000). Further, cannabinoids have been shown to reduce the size of malignant gliomas, a rare form of brain tumour, in rats (Galve-Roperh et al., 2000). In addition to these exciting new preclinical findings, cannabis has recently passed Phase 1 clinical trials in the United Kingdom. Thus, the therapeutic efficacy of medical marijuana use on patients will soon be decided (The Pharmaceutical Journal, 1999).

In light of the high prevalence of cannabis use and its recent therapeutic indications, basic research into the effects of cannabis on brain and behaviour is important. Specifically, a neuropharmacological understanding of the possible habit-forming nature of cannabis needs to be addressed. If cannabis is habit-forming in a subset of users, then its therapeutic potential may be restricted, as is currently the case with opiates. Conversely, if persons are becoming addicted to cannabis then anti-craving pharmacotherapies based on cannabinoid receptor antagonists may be effective in assisting users in their efforts to abstain from use, or to reduce the amount consumed. Finally, novel therapeutically useful cannabinoid compounds can only be preclinically tested for their abuse liability if a viable animal model of cannabis dependence is created. At present this is not a reality. However efforts to meet these objectives are strongly aided by spectacular recent advances in basic cannabinoid science.

1.3. Recent advances in cannabinoid science

"One will find in hashish nothing miraculous, absolutely nothing but an exaggeration of the natural. The brain and organisms on which hashish operates will produce only the normal phenomena peculiar to that individual -- increased, admittedly, in number and force, but always faithful to the original".

French poet, Charles Baudelaire, circa 1850.

In the last decade, remarkable advances have been made in the science of cannabinoids and the endogenous cannabinoid system (Mechoulam, Fride, & Di Marzo, 1998). The term "cannabinoid" originally was used to describe naturally occurring dibenzopyran chemicals found predominately in the leaves and flowering tops of the female cannabis plant (Science and Technology Committee, 1998). These classical cannabinoid receptor agonists include Δ^9 -tetrahydrocannabinol (Δ^9 -THC), Δ^8 -tetrahydrocannabinol, cannabinal and cannabidiol (Pertwee, 1997). Now the term "cannabinoid" also encompasses substances that occur endogenously in mammals such as anandamide, and synthetic cannabinoid receptor agonists, such as CP 55,940, which is used throughout the current thesis (see section 2.2.1.3.). See Figure 1.1. for the chemical structures of Δ^9 -THC and CP 55,940. CP 55,940 is an important compound as it was used in a tritiated form to isolate cannabinoid binding sites in the brain (Herkenham et al., 1991b) which foreshadowed the discovery of functional cannabinoid receptors (Devane, Dysarz, Johnson, Melvin, & Howlett, 1988).

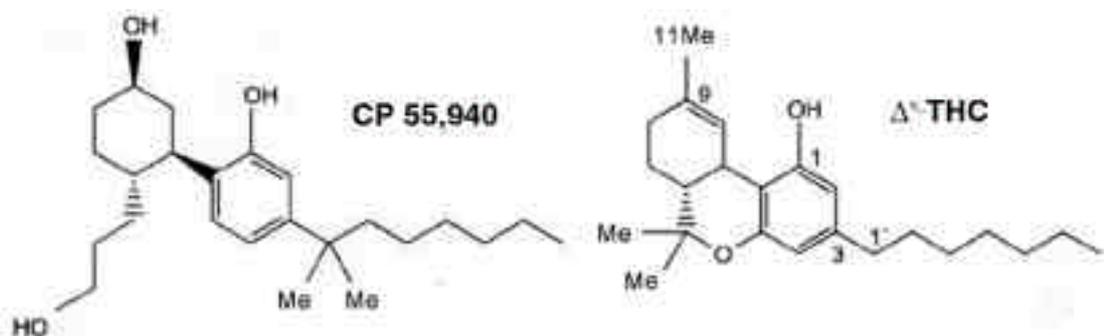


Figure 1.1. Chemical structures of the main psychoactive constituent of the cannabis plant, Δ^9 -THC, and its synthetic analogue, CP 55,940. Adapted from a pamphlet by Patricia Reggio on cannabinoid receptors supplied by Tocris.

The most important neuropharmacological event underlying the consumption of cannabis by humans appears to be the binding of Δ^9 -THC to brain cannabinoid CB₁ receptors. Membrane perturbation by Δ^9 -THC was thought to offer an alternative

mechanism of action to receptor activation. However, this explanation of the effects of Δ^9 -THC appears implausible because membrane perturbation does not occur at doses that are physiologically relevant (Howlett, 1995). Although the cannabis plant contains up to 60 cannabinoid substances (Adams & Martin, 1996; Dewey, 1986), Δ^9 -THC appears to be the one most responsible for the distinctive sensory disturbances, cognitive changes and euphoria associated with cannabis intoxication. Recent evidence indicates that human users will self-administer cannabis in a way that is related to its Δ^9 -THC content, smoking significantly fewer cigarettes devoid of Δ^9 -THC (Haney, Comer, Ward, Foltin, & Fischman, 1997). However, future research must also consider whether synergistic interactions occur between the different cannabinoid substances found within plant forms of cannabis. Unless this is pursued it cannot be ruled out that studying the effects of cannabis based on the administration of one cannabinoid substance (for example, Δ^9 -THC) may have little ecological validity.

Δ^9 -THC is highly lipophilic and readily crosses from blood brain barrier where it binds to CB₁ receptors. When bound with a cannabinoid receptor agonist these G_i or G_o protein-coupled receptors regulate cellular function by inhibiting calcium ion channels or adenylyate cyclase activity (Di Marzo, Melck, Bisogno, & De Petrocellis, 1998). Moreover, evidence exists which shows that CB₁ receptors may also regulate other signal transduction mechanisms in the cell (Pertwee, 1997). CB₁ receptors are widely distributed throughout the brain, with a particularly high density in the cortex, hippocampus, basal ganglia and cerebellum (Herkenham et al., 1991a,b; Pertwee, 1997; Pettit, Harrison, Olson, Spencer, & Cabral, 1998; Tsou, Brown, Sanudo-Pena, Mackie, & Walker, 1998). In the brain, many CB₁ receptors appear to be presynaptically located where they are thought to modulate the action and release of other neurotransmitters (Di Marzo et al., 1998; Pertwee, 1997). CB₁ receptors are also found in many different peripheral tissues. Peripheral tissues which contain the highest levels of levels CB₁ receptor protein are the pituitary gland and leukocytes (Pertwee, 1997).

Following the isolation of the CB₁ receptor another receptor subtype has been discovered. This is a splice variant of the CB₁ receptor called CB_{1(a)} (Shire et al., 1995). The deoxyribonucleic acid (DNA) that encodes this receptor is 61 amino acids shorter than the amino acid sequence that encodes the CB₁ receptor. Further, the first 28 amino acids of the CB_{1(a)} receptor are quite different to the corresponding coding region of the CB₁ receptor (Shire et al., 1995). This receptor is distributed similarly to the CB₁ receptor, however, at much lower levels (Pertwee, 1997). In addition, a peripherally distributed cannabinoid receptor has been discovered called the CB₂ receptor. This receptor has only 44% sequence homology with the CB₁ receptor (Munro, Thomas, & Abushaar, 1993). CB₂ receptors are G_{i/o} protein-coupled and are found mainly on cells of the immune system (for example, spleen, leukocytes and tonsils), with smaller levels in other tissues (such as the heart, lung and testes) (The Science and Technology Committee, 1998; Pertwee, 1997). As yet no CB₂ receptors have been found in the brain (Pertwee, 1997).

At least two putative endogenous ligands – arachidonyl ethanolamide (anandamide) and 2-arachidonylglycerol (2-AG) - are thought to bind to CB₁ receptors. Anandamide was discovered in 1992 (Devane et al., 1992), and has since been shown to be synthesised and released by neurons and astrocytes, and to be subject to high affinity reuptake processes and enzymatic degradation (Beltramo et al., 1997; Desarnaud, Cadas, & Piomelli, 1995; Di Marzo et al., 1994; Di Marzo et al., 1998). When injected into laboratory rats or mice, anandamide shows a constellation of classical cannabinoid effects including inhibition of locomotor activity, analgesia, memory impairment and hypothermia (Crawley et al., 1993; Fride & Mechoulam, 1993; Mallet & Beninger, 1996). The potency of anandamide can be greatly increased by drugs that inhibit its hydrolysis or block its reuptake, recent examples being oleamide (Mechoulam et al., 1997) and AM-404 (Beltramo et al., 1997) respectively.

The second endogenous cannabinoid ligand, 2-AG, was discovered in 1995 (Mechoulam et al., 1995) and has also been shown to be released by and synthesised in neurons (Stella, Schweitzer, & Piomelli, 1997). Further, like ⁹-THC and anandamide,

2-AG impairs long-term potentiation in the hippocampus (Stella et al., 1997). Interestingly, data suggest that 2-AG is some 170 times more abundant in the brain than anandamide (Stella et al., 1997), suggesting that it may have primacy as an endogenous cannabinoid receptor ligand. Supporting this, a recent study has shown that 2-AG has higher affinity for the CB₁ receptor than anandamide (Sugiura et al., 1999). However, contradictory findings show that anandamide, but not 2-AG, is released in the dorsal striatum in freely moving rats (Giuffrida et al., 1999). This paper also reported that anandamide and dopamine (DA) might functionally interact in striatal neural signaling.

Researchers have made progress in determining which populations of cannabinoid receptors mediate which aspect of cannabinoid action. Thus cannabinoid receptors located in the cortex and hippocampus may mediate the effects of cannabinoids on cognition and memory (Lichtman, Dimen, & Martin, 1995), while receptors in the periaqueductal grey (PAG) and spinal cord are implicated in the analgesic effects of the drug (Hohmann, Martin, Tsou, & Walker, 1995; Lichtman et al., 1995; Lichtman & Martin, 1991b; Walker et al., 1999). Positron emission tomography (PET) studies have indicated that the subjective feeling of intoxication following intravenous ⁹-THC is correlated with increases in glucose metabolism in the cerebellum and increased blood flow to the right frontal cortex of human participants (Mathew, Wilson, Coleman, Turkington, & DeGrado, 1997; Volkow et al., 1996).

However, as yet, there are few clues as to the critical neural substrates underlying the euphoria-inducing and possible habit-forming properties of cannabinoids. Based on the current evidence it appears plausible that the endogenous cannabinoid system could play some role in euphoria and addiction by modulating neurotransmitter action and release critical to these processes (Di Marzo et al., 1998).

1.4. Cannabis dependence in humans

"Marijuana can put a serious chokehold on long-term users who try to quit."

Donna Shalala, Secretary of Health and Human Services in the U.S.A., Wall Street Journal, 1995

Much controversy surrounds the clinical definitions of substance dependence and substance abuse (Helzer, 1994). Some view these constructs as being qualitatively distinct, while others conceptualise them as residing on the same continuum with substance dependence being a more extreme condition. In any case, the Diagnostic and statistical manual of mental disorders, 4th edition (DSM-IV) (A.P.A., 1994) definition of substance dependence contains some of the criteria that define substance abuse but has additional criteria. The diagnostic criteria for substance abuse solely encapsulates the negative social consequences that drug taking may have, for example, an inability to fulfil work commitments. Substance dependence, while still encapsulating these negative social consequences, also contains criteria based on recognised physiological and behavioural aspects of chronic drug use, such as tolerance, withdrawal and compulsion to use (Nathan, 1994).

The current thesis concentrates discussion on dependence because this diagnostic category is what non-human research on addiction attempts to model. In comparison to dependence syndromes associated with tobacco, alcohol or the opiates, the cannabis dependence syndrome is poorly understood. This stems from cannabis dependence only recently emerging as a clinical concern (Swift, Copeland, & Hall, 1998). For most of the 1960's and 1970's much research was conducted on alcohol and opiate dependence, while research surrounding cannabis dependence was sparse. At that time a cannabis dependence syndrome was thought unlikely to exist. In 1967, the American Council on Mental Health stated that a cannabis dependence syndrome had never been demonstrated (Graham, 1976). However, more recent data now suggest that such a syndrome *does* exist, and the symptomatology of cannabis dependence and the long-term effects of cannabis use need to be more adequately researched.

An increasing body of evidence suggests that significant numbers of long-term cannabis users are dependent upon the drug, according to the diagnostic criteria for drug

dependence presented by the American Psychiatric Association (DSM-III-R; DSM-IV) and World Health Organization (ICD-10) (see Table 1.1.). DSM-IV (A.P.A., 1994) defines seven diagnostic criteria for a cannabis dependence syndrome (see Table 1.1). To be diagnosed an individual must have experienced at least three of the seven criteria at any time within the previous 12 months. According to these criteria, it has been estimated that approximately 0.7% of the total U.S.A. adult population may be cannabis dependent (Kandel, Chen, Warner, Kessler, & Grant, 1997), with about 9% of persons who have used the drug meeting dependence criteria (Anthony, Warner, & Kessler, 1994).

While these statistics may be cause for concern, recent evidence highlights that both the concept of cannabis dependence, and the diagnostic tools used to assess it, need improvement. Evidence suggests that “cannabis dependence”, as assessed by different diagnostic tools, is not a unified, easily discernible or coherent phenomenon. The diagnosis of dependence with heavy long-term users of the drug varied between 39% and 92% depending upon the instrument being used for assessment (Swift, Hall, & Copeland, 1997). A lack of correlation across different diagnostic approaches has been reported in other studies (Compton, Cottler, Dorsey, Spitznagel, & Mager, 1996a,b; Cottler et al., 1997; Swift, Hall, Didcott, & Reilly, 1998). Thus, diagnostic tools used in cannabis research and clinical settings need to be improved. Improvements can be made either in research that more adequately defines cannabis dependence; or in studies which attempt to isolate the appropriate setting of diagnostic cut-offs (Swift, Copeland et al., 1998). For example, one study has provided possible reasons for why there is poor cross system agreement between the DSM-IV and ICD-10 criteria for cannabis dependence (Rounsaville, Bryant, Babor, Kranzler, & Kadden, 1993). One reason provided is the criterion of “a strong compulsion to use” which was included in ICD-10, yet is absent from DSM-IV. This criterion may be more important in defining cannabis dependence than previously realised. Further basic and clinical research may help to characterise this phenomenon.

Table 1.1. Criteria for substance dependence in DSM-IV and ICD-10 used as the basis for diagnosis of cannabis dependence in humans.

DSM-IV criteria	ICD-10 criteria
<p>A maladaptive pattern of substance use, leading to clinically significant impairment or distress, as manifested by three (or more) of the following occurring at any time in the same 12-month period:</p> <ol style="list-style-type: none"> 1) tolerance, as defined by either of the following: <ol style="list-style-type: none"> a) a need for markedly increased amounts of the substance to achieve intoxication or desired effect b) markedly diminished effect with continued use of the same amount of the substance 2) withdrawal as manifested by either of the following: <ol style="list-style-type: none"> a) the characteristic withdrawal syndrome for the substance (no specific listing for cannabis) b) the same (or a closely related) substance taken to relieve or avoid withdrawal symptoms 3) the substance is often taken in larger amounts or over a longer period than was intended 4) there is a persistent desire or unsuccessful efforts to cut down or control substance use 5) a great deal of time is spent in activities necessary to obtain the substance, use the substance or recover from its effects 6) important social, occupational, or recreational activities are given up or reduced because of substance use 7) the substance use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the substance 	<p>Three or more of the following manifestations should have occurred together for at least 1 month or, if persisting for periods of less than 1 month, should have occurred together repeatedly within a 12-month period:</p> <ol style="list-style-type: none"> 1) a strong desire or sense of compulsion to take the substance 2) impaired capacity to control substance-taking behaviour in terms of its onset, termination, or levels of use, as evidenced by the substance being often taken in larger amounts or over a longer period than intended, or by a persistent desire or unsuccessful efforts to reduce or control use; 3) a physiological withdrawal syndrome when substance is reduced or ceased, as evidenced by the characteristic withdrawal syndrome for the substance, or by use of the same (or closely related) substance with the intention of relieving or avoiding withdrawal symptoms; 4) evidence of tolerance to the effects of the substance, such that there is a need for significantly increased amounts of the substance to achieve intoxication or the desired effect, or a markedly diminished effect with continued use of the same amount of the substance; 5) preoccupation with substance use, as manifested by important alternative pleasures or interests being given up or reduced because of substance use; or a great deal of time spent in activities necessary to obtain, take or recover from the effects of the substance 6) persistent substance use despite clear evidence of harmful consequences, as evidenced by continued use when the individual is actually aware, of the nature and extent of harm.

Diagnosis of dependence appears to be linearly associated with frequency and quantity of cannabis use, with the highest rate of cannabis dependence diagnosed in last year marijuana users who used near daily or daily (Chen, Kandel, & Davies, 1997). In addition, an Australian study has shown that 92% of very frequent long-term cannabis users (regular use for 11 years and daily use in half of the sample) met DSM-III-R criteria for dependence (Swift et al., 1997). Higher rates of dependence in near daily users was observed in adolescents (ages 12-17) compared to adults (age 18+) with these rates declining with age (Chen et al., 1997). These data indicate that cannabis dependence may be a particular problem for individuals in crucial formative years. One reason for this is that chronic cannabis use may retard performance at school because of cognitive and learning deficits associated with cannabis intoxication (Solowij, 1995; Solowij, Michie, & Fox, 1995).

To put cannabis dependence in perspective, clinical data assessing relative severity of different substances of abuse indicates that cannabis has less abuse liability than other drugs of abuse, such as tobacco, heroin, cocaine or alcohol (Anthony et al., 1994; Rounsaville et al., 1993). In a sample of users who had used more than six times, more than 80% met dependence criteria when using tobacco, cocaine, or opiates, whereas only 42% met dependence for cannabis (Rounsaville et al., 1993). While cannabis may not have as great abuse liability as other drugs, significant numbers of cannabis users still seek help from general practitioners and mental health professionals to abstain from cannabis use (Copeland, Rees, & Swift, 1999; Stephens et al., 1993; Stephens, Roffman, & Simpson, 1994). These users commonly report that they find it very difficult to stop using the drug and that they have experienced memory loss and withdrawal symptoms (Stephens et al., 1993).

In addition, a high prevalence of long-term cannabis users report psychological problems (Stephens et al., 1993; Swift et al., 1997). Forty percent of long-term cannabis users, with regular use for 11 years and daily use in half of the sample, have consulted mental health professionals, been admitted into a psychiatric institution or prescribed medication to treat a psychiatric illness (Swift et al., 1997). However, it is

questionable whether this frequency significantly differs from the frequency of mental illness found in a similar sample of people who had not ever used recreational drugs. Unfortunately this vital control group was not included in either of the above studies. Furthermore, assuming that there is an increased frequency of mental disorder in long-term cannabis users, it is hard to determine whether this is a consequence of cannabis consumption or a consequence of a disorder pre-existing the consumption of cannabis. So while cannabis dependence may not have as high abuse liability as either cocaine or heroin (Woody, Cottler, & Cacciola, 1993), it is possible that long-term cannabis use, which is associated with a cannabis dependence syndrome, could have serious psychological and health implications (Hall & Solowij, 1998).

1.5. Why Do People Become Dependent On Cannabis?

Various social, economic and psychological factors may be important in determining why people become dependent on cannabis. While these factors also require research the current thesis provides a neuropharmacological focus. According to this focus, generally two explanations have been provided to account for why people become dependent. The first argues that a cannabis withdrawal syndrome is responsible for the long-term maintenance of cannabis use. The second argues that cannabis has effects on crucial motivational circuitry in the brain and through long-term drug use these brain areas are "usurped" or "short-circuited" so that the user becomes entrenched in cannabis seeking behaviour and consequent long-term cannabis use or abuse. These possible explanations of why people become dependent on cannabis should not be seen as mutually exclusive. While they provide different reasons for sustained cannabis consumption, both may simultaneously play an important role.

1.5.1. The cannabis physical withdrawal syndrome.

" Studies show that after abruptly stopping marijuana use, the long-term heavy pot user may develop signs and symptoms of withdrawal."

Neil Swan, National Institute of Drug Abuse
Notes, 1994.

During the 1960's and 1970's cannabis dependence was thought unlikely because cannabis did not appear to induce a withdrawal syndrome. At that time the physical withdrawal syndrome was construed as being central to addiction, and withdrawal syndromes had been characterized in chronic users of alcohol and opiates. However, recent evidence shows that a cannabis withdrawal syndrome can be elicited in humans and animals (Aceto, Scates, Lowe, & Martin, 1995, 1996; de Fonseca, Carrera, Navarro, Koob, & Weiss, 1997; Haney, Ward, Comer, Foltin, & Fischman, 1999a, 1999b; Jones, Benowitz, & Bachman, 1976; Tsou, Patrick, & Walker, 1995; Wiesbeck et al., 1996). This physical withdrawal syndrome is thought to be significant as it provides a reason for why cannabis users find it difficult to abstain from the drug. That is, abstinence from chronic use of cannabis gives rise to withdrawal symptoms and to avoid such aversive consequences, the individual relapses into using cannabis to alleviate these negative symptoms. This explanation derives from instrumental conditioning, specifically negative reinforcement, where a particular behaviour is made more likely when it is followed by the removal of an aversive stimulus (Mazur, 1998). Thus, for those dependent on cannabis, the difficulty in abstaining from use may, in part, derive from the need to alleviate the aversive consequences of abstinence.

The first anecdotal reports of a cannabis withdrawal syndrome were based on clinical observations of regular cannabis users who attempted to abstain from use (Bensusan, 1971; Dilsaver, Leckrone, & Greden, 1984; Fraser, 1949; Rohr, Skowlund, & Martin, 1989). However, observations made in these studies were not scientifically rigorous and were open to many alternative explanations including that the syndrome could merely be a product of withdrawal from other recreational drugs (Compton, Dewey, & Martin, 1990).

More recent studies have overcome these limitations by providing a more controlled environment where participants live in an experimental laboratory. Participants are closely monitored in this environment and Δ^9 -THC is the only drug that is provided (except tobacco). When a period of regulated cannabis consumption has ended the participants are evaluated for a cannabis withdrawal syndrome (Georgotas & Zeidenberg, 1979; Jones et al., 1976; Mendelson, 1976; Nowlan & Cohen, 1977). One of the best of these studies involved giving daily “elephant doses” (210 mg) of Δ^9 -THC to human volunteers over a 30 day period (Jones et al., 1976). Following cessation of the dosing regime, marked behavioural and subjective changes were noted, including hyperactivity, inner restlessness, sweating, anorexia and insomnia. In addition, these symptoms were reduced when Δ^9 -THC was re-administered (Jones, Benowitz, & Herning, 1981).

While the study by Jones et al. (1976) provides strong evidence for a cannabis withdrawal syndrome it became questionable whether a syndrome would occur in more typical conditions of cannabis use where much smaller doses are self-administered. In a recent residential study it has been shown that a cannabis withdrawal syndrome does occur even when lower doses of Δ^9 -THC are used (Haney et al., 1999a). In this study, withdrawal symptoms were evident in healthy marijuana users who were administered between 80 and 120 mg of Δ^9 -THC per day for a smaller period of time (4 days). The abstinence symptoms reported in this study are similar to the ones reported by Jones et al. (1976) and include negative affective states such as anxiety, irritability, depression, and negative physical ailments such as reduced quantity and quality of sleep, appetite suppression and gastrointestinal complaints. To add weight to this finding, a follow up study was conducted using a very similar experimental design except that marijuana cigarettes were administered rather than oral doses of Δ^9 -THC. This study again reported a cannabis withdrawal syndrome that was comprised of the same symptoms as reported in their previous study (Haney et al., 1999b). Thus, a cannabis withdrawal syndrome *does* exist which can be elicited under conditions in which excessive users would take cannabis in the natural ecology.

1.5.2. The role of cannabis withdrawal in maintaining cannabis dependence.

When weighing up the role of a cannabis withdrawal syndrome in maintaining chronic cannabis use it is important to weigh up two issues; the severity of the withdrawal syndrome and whether a cannabis withdrawal syndrome can satisfactorily account for all aspects of cannabis dependence. First, the cannabis withdrawal syndrome, while it exists, appears to be mild in comparison to the alcohol, benzodiazepine and heroin withdrawal syndromes (Abood & Martin, 1992; Haney et al., 1999a, 1999b). Thus, the assertion that cannabis withdrawal has a pivotal role in uncontrolled cannabis use is much less persuasive because the syndrome is less severe than those exhibited by other drugs of abuse.

To further downplay the role of a purely physical withdrawal syndrome as a comprehensive explanation of cannabis dependence is the observation that most of the physical symptoms of cannabis withdrawal subside after one week of abstinence (Georgotas & Zeidenberg, 1979; Jones et al., 1976; Wiesbeck et al., 1996). Thus, the physical symptoms (e.g. decreased ability to sleep and gastrointestinal upset) are only important, if at all, in sustaining chronic cannabis use in the short-term. What appears to be more important are the long-lasting psychological changes associated with long-term cannabis use, that is, anxiety, irritability, obsessive thoughts about cannabis use (craving) and the compulsion to put these thoughts into action. Therefore, it is possible that the sustained use of cannabis derives, not from seeking to avoid the mild and short-lived physical consequences of abstinence, but from the need of the chronic user to alleviate long-lasting abstinence-induced psychological consequences.

1.5.3. Cannabis withdrawal in laboratory animals.

"... 'precipitated withdrawal' is now cited as evidence that marijuana causes physical dependence. In fact, it has no relevance to human marijuana users who, upon ceasing use, always experience a gradual separation of THC from receptors"

Marijuana myths, marijuana facts, 1997.

Animal research attempting to illustrate a cannabis withdrawal syndrome through the abrupt termination of high doses of cannabinoids have not been able to establish any

clear consequential behavioural changes (Compton et al., 1990). However, the recent availability of a CB₁ receptor antagonist (SR 141716A) has allowed the elucidation of a precipitated withdrawal syndrome from cannabinoid receptor agonists in rats (Aceto et al., 1995; Tsou et al., 1995). In a typical experiment, rats are given daily high doses or continuous infusion of cannabinoids and then challenged with the cannabinoid CB₁ receptor antagonist SR 141716A. This produces a short behavioural syndrome in rats consisting of hyperactivity, scratching, face rubbing, paw-licking, wet dog shakes, ptosis (drooping of the eyelid) and convulsive signs such as forepaw fluttering and myoclonic spasms.

While clearly presenting a fascinating syndrome, there are doubts as to the exact relevance of SR 141716A-precipitated withdrawal. The long elimination half-life of ⁹-THC ensures that human users are never exposed to the dramatic and sudden reduction in CB₁ receptor activation that underlies the rat model. Notably, when rats are given a natural (i.e. non-precipitated) withdrawal from chronic high doses of cannabinoids, few, if any, of the behavioural or neurochemical signs of withdrawal observed when using SR 141716A are reported (Aceto et al., 1995, 1996; de Fonseca et al., 1997; Rubino et al., 1998; Tsou et al., 1995). In addition, there is also legitimate concern about the extent to which SR 141716A may cause abnormal effects regardless of prior exposure to ⁹-THC. This view is reinforced by evidence that indicates SR 141716A may be an inverse agonist, and thus has its own intrinsic effects upon the CB₁ receptor opposite to that of classical CB₁ receptor agonists (Bouaboula et al., 1997; Compton, Aceto, Lowe, & Martin, 1996c; MacLennan, Reynen, Kwan, & Bonhaus, 1998). For instance, in rats, SR 141716A given alone displayed powerful anxiogenic properties (Navarro et al., 1997) and induced signs of the withdrawal syndrome such as wet dog shakes, forepaw fluttering, hyperactivity and scratching (Aceto et al., 1996; Compton et al., 1996c; de Fonseca et al., 1997; Rubino et al., 1998).

A recent precipitated withdrawal study in rats has shown that only a moderate withdrawal syndrome follows termination of the chronic administration of CP 55,940 and an injection of SR 141716A. This moderate withdrawal syndrome was comprised

of merely chewing, turning and digging (Rubino et al., 1998). The incidence of wet-dog shakes, forepaw fluttering, and scratching, while they were observed, were deemed not to be associated with withdrawal from chronic cannabinoid administration because those same effects were observed in rats pretreated with vehicle and given SR 141716A (Rubino et al., 1998). So at this point, it is difficult to dissociate the precipitated withdrawal syndrome from the effects of the antagonist used to produce it. One possibly important test in verifying the applicability of the syndrome would be to determine whether rats would self-administer Δ^9 -THC during withdrawal. This experiment has yet to be done.

Other evidence has highlighted the neurochemical consequences of SR 141716A-precipitated withdrawal. An increase in the release of corticotropin releasing hormone (CRH) in the central nucleus of the amygdala (CEA) accompanies SR 141716A-precipitated withdrawal, an effect that is also seen during withdrawal from alcohol, cocaine and opiates, and which may be correlated with withdrawal-induced dysphoria (de Fonseca et al., 1997). Importantly, SR 141716A was shown to have no effect on CRH release when administered alone (de Fonseca et al., 1997).

Two other studies have also provided persuasive evidence that "normal" DA activity in the mesolimbic pathway becomes dependent on the administration of Δ^9 -THC after it has been chronically administered. First, chronic administration of Δ^9 -THC followed by a SR 141716A challenge reduced DA release in the shell of the nucleus accumbens (NAS) (Tanda, Loddo, & Di Chiara, 1999). It was also found that the SR 141716A did not affect DA release in this critical part of the mesolimbic system in rats that were pretreated with saline. Furthermore, another study has shown reduced DA activity in meso-accumbens neurons after cessation of chronic Δ^9 -THC administration (Diana, Melis, Muntoni, & Gessa, 1998). Most importantly, baseline DA levels were restored with the re-administration of Δ^9 -THC. The reductions in baseline DA levels were observed in the absence of any overt behavioural signs of withdrawal. These studies further reinforce the notion that physical withdrawal is less important than abstinence-induced dysphoria and craving in maintaining cannabinoid dependence. This

is true to the extent that mesolimbic DA circuitry may underly the psychological aspects of drug dependence.

1.5.4. Animal models of cannabinoid self-administration.

Researchers working on the neural basis of drug craving have been aided substantially by the fact that rats and monkeys will readily self-administer most recreational drugs that humans use including alcohol, nicotine, cocaine, amphetamine, heroin and phencyclidine (Bardo, 1998; Koob, 1996; Robinson & Berridge, 1993). This has allowed the use of animal subjects for extensive investigation of the neural and genetic basis of drug seeking behaviour and the development of novel pharmacotherapies for the treatment of drug craving.

Stemming from such research is the most compelling explanation for why individuals begin to habitually use drugs of abuse. This explanation, which still requires much research, generally shows that important motivational circuitry, or the mesolimbic DA system, guides the wanting and/or liking of natural rewards such as food, drink and sex, and this circuitry is "usurped" or "short-circuited" by chronic intake of drugs of abuse (Robbins & Everitt, 1999b; Robinson & Berridge, 1993). The consequence is that the user is "programmed" into obsessively seeking and taking drugs. It has been suggested that this "programming" arises from neuroadaptations that occur in response to the chronic intake of drugs. These neuroadaptations may "sensitize" the mesolimbic DA system, and thus the organism to the reinforcing effects of drugs (Robinson & Berridge, 1993). Additionally, it is thought that whenever the user becomes abstinent, the homeostatically dysregulated mesolimbic DA system mediates consequent drug craving and dysphoria, states which are alleviated by further drug-intake (Koob, 1996; Koob & Le Moal, 1997).

Unfortunately, despite more than 25 years of attempts, it can be argued that no satisfactory animal model of cannabis-seeking behavior has been derived. This has posed a major stumbling block for researchers interested in convincingly demonstrating the neural basis of cannabis dependence and also for researchers wishing to use a reliable model of abuse liability for testing new cannabinoid compounds. Repeated

attempts to obtain cannabis or cannabinoid self-administration in mice, rats and primates have been met with conspicuous failure (see Table 1.2.). Even rats and mice given months of forced-exposure to Δ^9 -THC decline to self-administer the drug when subsequently given a choice (Corcoran & Amit, 1974; Cutler, Mackintosh, & Chance, 1975; Leite & Carlini, 1974). While apparent intravenous Δ^9 -THC self-administration has been reported in rats (Takahashi & Singer, 1979, 1980, 1981), this appears to be little more than an artifact of a schedule induction procedure involving severe food restriction and the use of a concurrent non-contingent fixed-time delivery of food pellets. When the food pellets or food deprivation were removed from the experimental paradigm, the apparent self-injection of Δ^9 -THC disappeared (Takahashi & Singer, 1979).

There are many possible reasons why rats will not self-administer Δ^9 -THC. One possible reason is the pharmacokinetics of Δ^9 -THC. Unlike many drugs of abuse, the delayed onset of effect of Δ^9 -THC may make it more difficult for animals to associate intoxication with lever pressing in a self-administration paradigm. In addition, Δ^9 -THC's long elimination half-life, renders it unsuitable as a reinforcer where lever pressing across short test sessions is used as a dependent variable. Indeed, all drugs that are self-administered by rats or monkeys appear to have much shorter durations of action than Δ^9 -THC. Clearly, cannabinoids with a shorter duration of action such as anandamide should be tested for possible self-administration in rats and monkeys.

Another possible explanation of why rodents will not self-administer cannabis is based on the observation that Δ^9 -THC has predominately aversive effects on rodents (see Table 1.2.). That is, Δ^9 -THC may have greater effects upon neural substrates underlying aversion than those underlying reward. Thus, the rewarding properties of the drug are overshadowed by its aversive properties, which consequently rules out self-administration. The aversive effects of cannabinoid receptor agonists in rodents are well established. Several studies have shown that rats will learn to avoid an environment that is associated with administration of cannabinoids such as Δ^9 -THC or

Table 1.2. Studies of the reinforcing and anxiogenic effects of cannabinoids in laboratory animals

Model	Species	Substance	Effect	Reference
Self-administration	rat (W)	hashish, cannabis	-	(Corcoran & Amit, 1974; Leite & Carlini, 1974)
		⁹ -THC	+ ^a	(Takahashi & Singer, 1979, 1980, 1981)
	mouse (CD1)	WIN 55,212-2	+ ^b	(Ledent et al., 1999; Martellotta, Cossu, Fattore, Gessa, & Fratta, 1998)
	mouse (CFW)	cannabis	-	(Cutler et al., 1975)
	rhesus monkey	CP 55,940, ⁹ -THC, levonantradol nantradol	-	(Harris, Waters, & McLendon, 1974; Mansbach, Nicholson, Martin, & Balster, 1994; Young, Katz, & Woods, 1981)
Conditioned place preference	rat (Lew, S-D)	⁹ -THC	-	(Parker & Gillies, 1995; Sanudo-Pena et al., 1997)
	rat (L-E)	⁹ -THC	+/ O ^c	(Lepore et al., 1995)
	rat (W)	WIN 55,212-2	-	(Chaperon, Soubrie, Puech, & Thiebot, 1998)
	rat (W)	CP 55,940	-	(McGregor, Issakidis, & Prior, 1996c)
	mouse (CD1)	⁹ -THC	+/ O / ^{-c}	(Valjent & Maldonado, 2000)
ICSS enhancement	rat (L-E)	nabilone, ⁹ -THC	O	(Stark & Dews, 1980)
	rat (CDF)	levonantradol	O	(Kucharski, Williams, & Kornetsky, 1983)
	rat (Lew)	⁹ -THC	+	(Gardner et al., 1988; Lepore, Liu, Savage, Matalon, & Gardner, 1996a)
	rat (F, L-E, S-D)	⁹ -THC	O	(Lepore et al., 1996a)
Elevated plus-maze	rat (S-D)	⁹ -THC	-	(Onaivi et al., 1990)
	mouse	⁹ -THC	-	(Onaivi et al., 1990)
		nabilone	+	(Onaivi et al., 1990)
	rat (W)	cannabidiol	+	(Guimaraes, de- Aguiar, Mechoulam, & Breuer, 1994)
	mouse (C57BL/6)	⁹ -THC	O	(Onaivi et al., 1996)
	mouse (DBA/2)	⁹ -THC	O	(Onaivi et al., 1996)
	mouse (ICR)	⁹ -THC	-	(Onaivi et al., 1996)
Light-dark emergence	rat (W)	⁹ -THC, HU-210	-	(Rodriguez de Fonseca et al., 1996, 1997; Navarro et al., 1993)
	mouse	anandamide	O	(Crawley et al., 1993)
Ultrasonic vocalization	rat pups (L-E)	CP 55,940	+	(McGregor, Dastur, McLellan, & Brown, 1996b)

Footnotes:

^a Self-administration occurred only when rats were food-deprived and FI-1 food delivery schedule used.

^b Self-administration was only demonstrated for one test session.

^c Direction of effect depended on dose.

For place conditioning studies, "+" indicates a place preference, "O" indicates no effect, and "-" indicates a place avoidance.

For EPM, light-dark emergence and USV models, "+" indicates an anxiolytic effect, "O" indicates no effect, and "-" indicates an anxiogenic effect.

Abbreviations:

F: Fischer 344; ICSS: intracranial self-stimulation; L-E: Long-Evans; Lew: Lewis; W: Wistar; S-D: Sprague-Dawley; ⁹-THC: ⁹-tetrahydrocannabinol

its synthetic derivatives such as CP 55,940, a phenomenon known as conditioned place aversion (see Table 1.2.). Although, two studies have shown a conditioned place preference with Δ^9 -THC across a narrow dose range (Lepore, Vorel, Lowinson, & Gardner, 1995; Valjent & Maldonado, 2000), an overwhelming number of studies have failed to show such an effect (see Table 1.2.).

Valjent and Maldonado (2000) have recently provided compelling reasons for why the rewarding effects of Δ^9 -THC have been scarcely measured. This study lends credence to both of the aforementioned hypotheses. That is, this study is consistent with the effects of cannabinoids being predominately aversive because the aversive effects of cannabinoids outweigh the rewarding effects. It is also consistent with the notion that the rewarding effects of cannabinoids are difficult to experimentally delineate because Δ^9 -THC has a long duration of action (Valjent & Maldonado, 2000). Specifically this study showed that Δ^9 -THC could produce conditioned place preference or aversion dependent on the dose or experimental design employed. The strongest preference for the compartment paired with an injection of 1 mg/kg of Δ^9 -THC was revealed when mice were both, pre-exposed to an injection of Δ^9 -THC in the homecage before place conditioning, and when the duration of action of Δ^9 -THC was shortened by the administration of the cannabinoid receptor antagonist SR 141716A. However, typical conditioned place aversion to Δ^9 -THC was demonstrated when mice were administered 5 mg/kg of Δ^9 -THC without the previously mentioned experimental manipulations. Thus, a rewarding effect of Δ^9 -THC may be discerned by reducing the initial aversive effects of Δ^9 -THC, by using a smaller dose and by making the effects of Δ^9 -THC more temporally discrete.

The predominantly aversive effects of cannabinoids may well reflect an anxiogenic quality of these drugs. Surveys with humans have illustrated that cannabis users commonly report experiences of anxiety, panic and fear when intoxicated by the drug (Annis & Smart, 1973; Fishman, Rosenbaum, Yabusaki, & Carr, 1988; Hall, 1995; Hall & Solowij, 1998; Thomas, 1996; Weil, Zinberg, & Nelsen, 1968; Zuardi, Shirakawa, Finkelford, & Karniol, 1982). Approximately one in five human cannabis

users surveyed reported anxiety when smoking or ingesting cannabis (Annis & Smart, 1973; Thomas, 1996). Anxiety constitutes one of the adverse mental health effects associated with acute use of cannabis (Hall, 1995; Hall & Solowij, 1998). Not surprisingly, the anxiogenic response to cannabinoids in humans is strongly associated with subsequent discontinuation of cannabis use (Thomas, 1996).

In animal studies anxiogenic effects of cannabinoids have also been reported. In the elevated plus-maze (EPM) test, Δ^9 -THC reduced the amount of time spent on the open arms of the apparatus in both rats and mice suggesting an anxiogenic effect (Onaivi, Chakrabarti, Gwebu, & Chaudhuri, 1996; Onaivi, Green, & Martin, 1990). This reasoning is supported by the observation that the effect of Δ^9 -THC in the EPM is reversed by the anxiolytic diazepam (Onaivi et al., 1990). Similarly, in the light-dark emergence test, the synthetic cannabinoid receptor agonist HU-210 increased the emergence latency to a familiar open field and the mean time spent avoiding that field (Rodriguez de Fonseca, 1997; Rodriguez de Fonseca et al., 1996).

However, in contrast to the study by Valjent et al. (2000) mentioned earlier in this section, a recent study using a non-elevated plus maze model of anxiety has shown that the anxiogenic effects of the HU-210 in rats were enhanced with repeated exposure rather than diminished (Giuliani, Ferrari, & Ottani, 2000). However, it is still possible that species differences exist in the effects of cannabinoids, with mice being more susceptible to the rewarding effects, or less susceptible to the anxiogenic effects, in comparison to rats.

1.5.5. Cannabinoid self-administration in mice? Evidence and limitations.

A recent study has shown that mice self-administer the synthetic cannabinoid receptor agonist, WIN 55,212-2, at low doses under a continuous reinforcement schedule (CRF). The self-administration was blocked by SR 141716A, indicating that the behaviour was specifically mediated through CB₁ receptors (Martellotta et al., 1998). This finding is further validated by another study showing that knockout mice lacking the CB₁ receptor would not self-administer WIN 55,212-2 or morphine, unlike

wild type mice (Ledent et al., 1999). This finding has offered some hope to behavioural pharmacologists who are interested in creating an animal model to screen the abuse liability of new cannabinoid compounds. Unfortunately, the significance of this apparently successful demonstration of cannabis self-administration remains unclear. One alternative reason for this apparent cannabinoid self-administration is based on the unique experimental model of self-administration that was used. First, the response upon which the administration of WIN 55,212-2 was contingent was a nose-poke rather than the conventionally used bar-press. Thus, by using a response that is in the rodent's natural repertoire it may have been easier to demonstrate self-administration. However, the obvious disadvantage is that false positives are more likely. For a rodent to bar-press it must go beyond its natural repertoire, which clearly illustrates to the experimenter that the animal is motivated to gain the reward.

Secondly, the model used only provides for acquisition of a self-administration habit, not maintenance. Thus, mice were only given a single session in which they demonstrated self-administration. The maintenance of self-administration (i.e. stable self-administration across days or weeks of testing) is thought to be a more valid model of human drug dependence, because long-term use, and the motivation for prolonged use, are central characteristics of drug addiction. Notably, rats lever pressing for intravenous cocaine or heroin typically require 5-10 days of training before stable self-administration is achieved (Campbell, Lac, & Carroll, 1999; Hudzik, Wessinger, & McMillan, 1993; Lynch & Carroll, 1999; Roberts & Andrews, 1997). Because self-administration was only illustrated in one experimental session in the studies of Martelotta et al (1998) and Ledent et al (1999), it is possible that responses made could merely reflect inquisitive responding to subtle effects of the drug that were hedonically neutral. That is, novel experience in itself has been found to initiate instrumental responding. For example, rats will learn to bar press a lever when this response is contingent on the illumination of a novel light that arguably has no hedonic value (Berlyne, 1969; Berlyne & Koenig, 1965; Berlyne, Koenig, & Hirota, 1966).

Therefore, for this demonstration to be regarded as a robust one, maintenance of responding should be assessed over many sessions rather than just one.

It must also be noted that Martelotta et al (1998) and Ledent et al (1999) did not include the prototypical cannabinoid receptor agonist, Δ^9 -THC, in their studies. This begs the question of whether they failed in an attempt to train mice to self-administer Δ^9 -THC or that no attempt was made. Hopefully, future data will be forthcoming on this issue.

Clearly, further research should attempt to establish the reasons responsible for the promising demonstration of the acute positive reinforcing effects of WIN 55,212-2 and also extend this finding to a robust model that includes ongoing maintenance of cannabinoid self-administration.

1.5.6. Cannabinoid effects on the mesolimbic DA system.

Δ^9 -THC, like heroin, alcohol, nicotine and cocaine, increases levels of extracellular DA in the NAS of rats. The NAS is an output of the mesolimbic DA system and has been shown to be critical to the rewarding properties of intracranial self-stimulation, drugs of abuse and natural reinforcers (Bardo, 1998; Koob, 1996; Koob & Le Moal, 1997; Robbins & Everitt, 1999b; Robinson & Berridge, 1993; Wise, 1996).

The finding that cannabinoids increase DA efflux in the NAS, was obtained using the technique of microdialysis, and was first made by Gardner and colleagues (Chen et al., 1990; Chen, Paredes, Lowinson, & Gardner, 1991; Gardner & Lowinson, 1991). Subsequently, it has been verified that this effect is mediated by CB₁ receptor activation, and that it is also dependent on μ_1 opioid receptors (Tanda, Pontieri, & Di Chiara, 1997). Furthermore, it was demonstrated that DA efflux was enhanced in the shell of the NAS, a subregion which appears to be specifically related to the rewarding effects of drugs, but not in the core, a region more associated with control of motor behaviour (Johnson, Goodman, Condon, & Stellar, 1995; Pontieri, Tanda, & Di Chiara, 1995).

The mechanism underlying increased DA efflux in the shell of the NAS may involve Δ^9 -THC increasing the firing rate of ventral tegmental area (VTA) neurons

(French, 1997; French, Dillon, & Wu, 1997). The cell bodies of the mesolimbic DA system are found in the VTA and these neurons extend to dendritic outputs that are found in the NAS. However, some evidence conflicts with the finding that cannabinoids increase VTA firing rate (Gifford, Gardner, & Ashby, 1997) and uncertainty surrounds the possible role of VTA μ_1 opioid receptors (French, 1997; Tanda et al., 1997).

Whether it is true or false, the capacity of a drug to increase DA efflux in the NAS has long been taken as an index of its abuse potential, according to the classic "DA theory of reward" (Robinson & Berridge, 1993; Wise & Bozarth, 1987). However, this classic theory is not aging well in the light of new evidence. First, it is clear that stressful procedures such as footshock, restraint, tail-pinch and social stress can increase DA efflux in the NAS yet clearly such events are not rewarding or habit forming (Di Chiara, Loddo, & Tanda, 1999; Doherty & Gratton, 1996; Joseph, Young, & Gray, 1996; Miczek, Mutschler, van Erp, Blank, & McInerney, 1999; Rouge-Pont, Deroche, Le Moal, & Piazza, 1998; Takahashi, Takada, Nagai, Urano, & Takada, 1998; Wu, Yoshida, Emoto, & Tanaka, 1999). Secondly, drugs that are not self-administered but have clear anxiogenic properties, such as FG 7142 and β -CCE, also increase DA efflux in the NAS (Horger, Elsworth, & Roth, 1995; McCullough & Salamone, 1992; Murai et al., 1994; Murai et al., 1998). Thus, the Δ^9 -THC-mediated increase in NAS DA is equally consistent with it being anxiogenic or rewarding. Thirdly, even with clearly addictive drugs such as cocaine and heroin, the correlation between increases in NAS DA and drug self-administration is complex and non-linear (Gratton, 1996). Thus, the first time a rat self-administers cocaine, DA levels in the NAS may decrease. Similarly, after the second or third self-administration of heroin, subsequent injections are associated with decreasing, rather than increasing DA levels in the NAS (Gratton, 1996). Finally, more recent analyses of what DA "does" in the NAS cite a possible role in attentional processes (Berridge & Robinson, 1998; Gratton, 1996; Ikemoto & Panksepp, 1999; Joseph et al., 1996; Robinson & Berridge, 1993; Schultz,

1998). In simple terms, the release of DA in the NAS may increase to any stimulus (either pleasant or aversive) that is of innate or learned importance to the animal.

Without a robust animal model of cannabis self-administration it has been impossible to convincingly show that the mesolimbic DA system is involved in the euphoria producing and habit-forming effects of cannabis. That is, no studies have been able to show a causal relationship between activation of mesolimbic DA circuitry and actual self-administration of cannabis in animals. Therefore, much confusion abounds in the literature over the exact nature of electrophysiological and neurochemical studies which show that cannabinoids do affect mesolimbic DA circuitry (McGregor, 1997).

While it is clear that mesolimbic DA circuitry is related to the self-administration of other commonly abused drugs, it is also clear that some aversive states are related to activation of this system. Therefore, it could equally be argued that activation of the mesolimbic DA system by cannabinoids merely reflects the clear aversive effects this drug has on rodents (see Table 1.2.). In light of the complex, multifaceted role of the mesolimbic DA system in controlling emotion and behaviour, only studies clearly demonstrating that manipulations of this system affect the self-administration of cannabinoids can be taken as firm evidence. This obviously lends credence to the view that a robust animal model of cannabis self-administration requires development.

1.6. Genetic basis of cannabinoid euphoria or dysphoria: the Lewis strain of rat

Building a reliable model of cannabinoid self-administration might be assisted by utilising specific rodent strains that are more vulnerable to the rewarding effects of drugs of abuse. This follows human research showing that certain individuals may have a genetic predisposition to becoming substance dependent. When looking specifically at human research on cannabis, data suggest that whether cannabis is experienced as euphoric or dysphoric may at least partly be determined by genetic factors. A recent twin study (Lyons et al., 1997) has shown that monozygotic twins are more likely than

dizygotic twins to be concordant in their emotional response (whether it be pleasant or unpleasant) to cannabis.

Furthermore, strain differences have been found in the rewarding effects of cannabis in rats (Chen et al., 1991; Gardner, 1998; Gardner & Lowinson, 1991; Lepore et al., 1996). When tested in the self-stimulation paradigm, Lewis rats showed a reduction in the current threshold for self-stimulation after acute Δ^9 -THC administration, suggesting a rewarding effect of this cannabinoid (Lepore et al., 1996a). No such effects were seen in Fischer 344 rats and only a marginal facilitatory effect was seen in Sprague-Dawley rats (Lepore et al., 1996a). In addition, Lewis rats were demonstrated to have enhanced DA efflux in the NAS in comparison to Sprague-Dawley rats when acutely administered Δ^9 -THC (Chen et al., 1991). The current thesis utilises Lewis strain rats in studies that probe the neuropharmacological underpinnings of cannabis reward and anxiety.

The genetic vulnerability of Lewis rats to the rewarding effects of recreational drugs is not limited to the cannabinoids. Lewis rats are also found to have a higher vulnerability to the rewarding effects of other drugs of abuse. For example, Lewis rats more avidly self-administer cocaine, morphine and ethanol and show a greater conditioned place preference to cocaine when compared to Fischer 344 rats (Kosten, Miserendino, Chi, & Nestler, 1994; Kosten et al., 1997; Suzuki, George, & Meisch, 1988a; Suzuki, Otani, Koike, & Misawa, 1988b). Lewis rats are also more susceptible to stimulant-induced behavioural sensitization when compared to Fischer 344 rats (Camp, Browman, & Robinson, 1994; Kosten et al., 1994; Ortiz, DeCaprio, Kosten, & Nestler, 1995).

These effects may rest on striking differences between Lewis rats and Fischer 344 rats in the biochemistry and electrophysiology of the mesolimbic DA system. For instance, Lewis rats appear to have more tyrosine hydroxylase (TH), an enzyme responsible for the synthesis of DA, per neuron in the VTA (Beitner-Johnson, Guitart, & Nestler, 1991; Harris & Nestler, 1996), and less TH and inhibitory G proteins in the NAS (Beitner-Johnson et al., 1991; Brodtkin et al., 1998). Furthermore, Lewis rats

also exhibit less basally active VTA neurons with a higher percentage of burst firing patterns (Minabe, Emori, & Ashby, 1995) and longer-lasting increases of DA release in the NAS following a cocaine challenge (Strecker, Eberle, & Ashby, 1995). The current thesis attempts to extend earlier behavioural and biochemical research to understand the neurobiological bases of the distinct effects that cannabinoids have on the Lewis rat.

The claim that Lewis rats find cannabinoids rewarding is controversial since a recent study documented a conditioned place aversion to Δ^9 -THC in these rats (Parker & Gillies, 1995). However, upon closer inspection this may not present a problem. Most drugs of abuse have aversive as well as rewarding components. For example, in humans and rats, both rewarding and aversive effects of cocaine have been reported (Benowitz, 1992; Blanchard et al., 1998; Ettenberg & Geist, 1991; Mantsch & Goeders, 1998; Rogerio & Takahashi, 1992; Williamson et al., 1997; Yang, Gorman, Dunn, & Goeders, 1992). Furthermore, an elegant study has shown that while rats avidly self-administer cocaine, they will also show signs of the anxiogenic effects of the drug which is correlated with retreats from self-administration (Ettenberg & Geist, 1991). At certain points in time the rats would stop and retreat from self-administering cocaine, a phenomenon that was dose-dependently reversed by diazepam (Ettenberg & Geist, 1991). Thus, the fact Lewis rats were reported to find cannabinoids at once rewarding and aversive is not necessarily problematic.

One of the most important questions to be answered is whether in Lewis rats the rewarding effects overshadow the aversive effects of cannabis and whether in other rat strains the aversive effects outweigh the rewarding effects. Recent evidence provides a mechanism whereby Lewis rats may find cannabinoids less aversive, stressful or anxiogenic. This mechanism generally relies on Lewis rats being less susceptible to stress. Consonant with this, the hypothalamo-pituitary-adrenal (HPA) axis of Lewis rats is hyporesponsive to various stimuli and releases less basal and stress-induced corticosterone in comparison to other rat strains such as Wistar rats (Brodkin et al., 1998; Chaouloff, Kulikov, Sarrieau, Castanon, & Mormede, 1995; Dhabhar, McEwen, & Spencer, 1993, 1997; Dhabhar, Miller, McEwen, & Spencer, 1995; Oitzl, van

Haarst, Sutanto, & de Kloet, 1995; Rivest & Rivier, 1994; Shurin, Kusnecov, Riechman, & Rabin, 1995; Sternberg et al., 1989a; Sternberg et al., 1989b). This appears to be a consequence of a deficiency in the ability of the hypothalamus to synthesise and secrete CRH in Lewis rats (Sternberg et al., 1989a; Sternberg et al., 1989b). In support of this, the paraventricular nucleus of the hypothalamus (PVN) of Lewis rats contain lower levels of CRH messenger ribonucleic acid (mRNA) than Wistar rats (Oitzl et al., 1995). Cannabinoids activate the HPA axis (Murphy, Munoz, Adrian, & Villanua, 1998; Weidenfeld, Feldman, & Mechoulam, 1994; Wenger, Jamali, Juaneda, Leonardelli, & Tramu, 1997) and it has also been demonstrated that CRH is critical to cannabinoid-induced anxiety (Rodriguez de Fonseca et al., 1996). Pre-exposure to the CRH receptor antagonist D-Phe CRF₁₂₋₄₁ reversed the anxiety-like behaviour of rats administered the cannabinoid receptor agonist HU-210. Therefore, cannabinoid-induced HPA activation and release of CRH may be deficient in Lewis rats, which provides a possible mechanism whereby Lewis rats are less susceptible to cannabinoid-induced anxiety.

1.7. “Gateway” effects of cannabis: the cross-sensitization phenomenon

"Since marijuana use, harmful as it is in its own right, is often a prelude to the use of other drugs...[it is] doubly disastrous."

Senator Orrin Hatch speaking at the U.S.A. Senate Judiciary Committee Hearing on teenage drug use, 1996.

The "gateway" theory, a phenomenon often alleged by politicians, holds that cannabis use may predispose users to the use of other "harder" drugs of abuse, such as heroin and cocaine. This theory generally comes in "hard" and "soft" versions. The "hard" version holds cannabis use invariably leads to an increase in the use of other harder drugs. The "soft" version, however, contends that cannabis use sometimes leads to an increase in the use of other harder drugs.

At first glance, research on the epidemiology of substance use in humans provides presumptive evidence that cannabis use is a "gateway" to the use of other

harder drugs of abuse. In Australia, Donnelly and Hall (1994) reported that the probability of having used heroin was 30 times higher for cannabis users than non-users (Donnelly & Hall, 1994). However, data still showed that some 96% of cannabis users had never used heroin. Similarly, the 17% of U.S. cannabis users who have tried cocaine is much higher than non-users, but still implies that 5 out of 6 cannabis users do not try cocaine (Wren, 1997). More recent Australian data suggest that long-term heavy cannabis users demonstrate very high levels of cigarette smoking, problem drinking and amphetamine use (Swift et al., 1997). While all these associations are interesting, none establish a temporal sequence where the use of cannabis is invariably a forerunner to the use of harder drugs of abuse.

The most informative and extensive research pertinent to the gateway theory are longitudinal studies conducted by Kandel and colleagues (Kandel & Yamaguchi, 1993; Kandel & Davies, 1997; Kandel, Yamaguchi, & Chen, 1992). These researchers have uncovered a highly predictable sequence of drug use in American adolescents which starts with alcohol and tobacco, is followed by cannabis (which is almost invariably the first illicit drug used) then hallucinogens and tranquilisers, before finally moving to cocaine or heroin. If anything, then, the primary "gateway" drugs are nicotine and alcohol. Interestingly, data show that cannabis use declines precipitously after the age of 25 with most previous cannabis users having ceased use by the age of 35 (Chen & Kandel, 1995; Kandel & Yamaguchi, 1993). However, unlike cannabis, the proportion of the population using licit drugs, such as alcohol and cigarettes, is maintained up until the age of approximately 50 (AIHW, 1998).

A criticism of those who use a "hard" version of the gateway theory to explain the aforementioned epidemiological data is that these data cannot establish a causal association between cannabis use and subsequent use of other drugs. That is, these studies cannot effectively illustrate that cannabis use invariably leads to an increase in the use of other harder drugs. The "gateway theory", in this form may not be a theory at all, but just a description of the typical sequence in which polydrug users begin using drugs of high prevalence (for example, cannabis) versus drugs of low prevalence (for

example, cocaine) (AIHW, 1998; Zimmer & Morgan, 1997). Misinterpretation of the statistical relationship between cannabis use and the subsequent use of harder drugs arises because the relationship observed has not been put into correct perspective. That is, statistical relationships, such as this, would exist between any common and uncommon activity. For example, most people who initiate surfboard riding (a rare activity) had previously swum in a swimming pool (a common activity). However, this does not mean that swimming causes surfboard riding. It simply means that people who can swim are more likely to initiate surfboard riding in comparison to people who do not swim. Similarly, people who have taken cannabis are more likely to try cocaine, but prior cannabis use does not cause cocaine use (Zimmer & Morgan, 1997).

While the "hard" version of the putative cannabis "gateway" theory is easy to discount, the "soft" version is not. This soft version, which holds that cannabis use sometimes leads to an increase in the use of other harder drugs, might function in many ways. One possibility is that cannabis use may expose human users to a social "gateway" where other harder drugs become more accessible via the criminal social nexus through which cannabis is obtained. This is the fundamental premise that has underpinned the de facto decriminalization of cannabis in The Netherlands where the dissociation of cannabis supply from heroin supply has been a primary aim. An alternative explanation comes from observations that both humans and animals differ greatly in their genetic predisposition to addiction in general (Blum, Cull, Braverman, & Comings, 1996; Piazza & Le Moal, 1996). Thus, the correlation between heavy cannabis use and tobacco smoking, problem drinking and amphetamine use may reflect no more than a global vulnerability to addiction in these persons. A third possible explanation, that does not necessarily exclude the first two, is that cannabis use may sensitize the brain substrate of "addiction" so that the user has a greater risk of becoming dependent on another substance (Robinson & Berridge, 1993). This would arise because other substances also derive their habit-forming properties from activating a common neural substrate (Koob, 1996; Koob & Le Moal, 1997; Robbins & Everitt, 1999b; Robinson & Berridge, 1993). The current thesis will address the later two

explanations by examining whether different rat strains when pre-exposed to cannabinoids are sensitized to the effects of cocaine.

Cannabinoid-mediated increases in DA efflux in the mesolimbic DA pathway, have been cited as evidence that cannabis use may predispose users to the addictive properties of other “harder” drugs (Tanda et al., 1997; Wickelgren, 1997). As previously argued, this is problematic because it is impossible, at the present time, to unambiguously relate DA efflux in the NAS to the self-administration of cannabis in rodents. However, if one assumes that the mesolimbic DA system is important for the self-administration of drugs, it is still only long-term changes in this system which offer a plausible model. That is, drug addiction is a chronic relapsing disorder that is never acquired in one trial. Long-term drug use and the associated long-lasting neural adaptations that “shortcircuit” or “usurp” this naturally important motivational system are thought to provide a possible explanation for why drug abusers frequently relapse after discontinuing use. In the field of neuropharmacology, sensitization of the mesolimbic DA system is thought to herald the expression of such neural adaptations. Following the neural model (Robinson & Berridge, 1993), repeated intermittent exposure to drugs such as cocaine, amphetamine, nicotine or heroin produces progressively greater drug-induced increases in DA efflux in the NAS, a phenomenon referred to as neurochemical sensitization (Fiorino & Phillips, 1999). This is coupled to a progressively greater behavioural response to the drug (behavioural sensitization) and increased positively reinforcing effects of the drug and drug-related cues (incentive sensitization). Interestingly, pre-exposure to one drug may also sensitize rats to the behavioural, neurochemical and incentive effects of another drug (cross-sensitization) and it is this phenomenon that gives the best animal model of the “gateway” effects presumed to occur in humans (Robinson & Berridge, 1993).

While in the past no clear link between sensitization and self-administration had been established in animal studies, recent evidence suggests the previously assumed association is justified. First, rats who show a predisposition to self-administering amphetamine also show an enhanced susceptibility to develop context-specific

sensitization (Jodogne et al., 1994). Further, self-administration of cocaine in rats has been demonstrated to induce behavioural sensitization as measured by locomotor activity (Phillips & Di Ciano, 1996). Moreover, recent studies reinforce the role of sensitization as a long-lasting or persistent neuroadaptation that mediates the relapse of drug use. These studies have shown that, after a prolonged drug-free period, drug-seeking behaviour in rats was reinstated, and this was associated with the expression of behavioural sensitization (De Vries, Schoffelmeer, Binnekade, Mulder, & Vanderschuren, 1998; De Vries, Schoffelmeer, Binnekade, & Vanderschuren, 1999; Mendrek, Blaha, & Phillips, 1998; Vanderschuren, Schoffelmeer, Mulder, & De Vries, 1999).

Thus, behavioural sensitization studies provide a route for examining the habit-forming nature of cannabis. Importantly, almost no research exists that attempts to test whether cannabis can cause behavioural sensitization or whether chronic cannabinoid pre-exposure can "cross-sensitize" the effects of other drugs of abuse. In fact, only one relevant study, apart from the one that describes work included in this thesis (Arnold, Toppo, Hunt, & McGregor, 1998), has been published. This study illustrated that chronic pre-treatment with Δ^9 -THC sensitized the locomotor-activating and stereotypy-inducing effects of amphetamine (Gorriti, Rodriguez de Fonseca, Navarro, & Palomo, 1999). However, while Gorriti and colleagues (1999) illustrated "cross-sensitization", they did not demonstrate behavioural sensitization to the effects of repeated administration of Δ^9 -THC in itself.

1.8. The role of the endogenous cannabinoid system in reward

Exogenous cannabinoids, such as Δ^9 -THC, have been shown to modulate the rewarding effects of electrical brain stimulation and increase the release of DA in the reward relevant NAS (Chen et al., 1990; Chen et al., 1991; Gardner, 1998; Gardner & Lowinson, 1991; Gardner et al., 1988; Tanda et al., 1997). These effects presumably occur because the endogenous cannabinoid system is involved in the neural substrate of reward.

Recent evidence indicates that this presumption is justified because the endogenous cannabinoid system has been shown to be critical to the rewarding effects of drugs of abuse and other reinforcers. Mice with targeted deletion of the cannabinoid CB₁ receptor gene exhibit reduced self-administration of morphine (Ledent et al., 1999). Furthermore, the selective cannabinoid CB₁ receptor antagonist SR 141716A blocks the acquisition of conditioned place preference to cocaine and morphine in rats (Chaperon et al., 1998). SR 141716 also reduces sucrose, ethanol, and beer consumption in rats suggesting involvement of the endogenous cannabinoid system in appetite and alcohol preference (Arnone et al., 1997; Colombo et al., 1998; Gallate & McGregor, 1999). Thus endogenous cannabinoids such as anandamide and 2-AG may play a key role in brain reward systems. This may be achieved by the endogenous cannabinoid system modulating neurotransmitter action and release which is critical to rewarding processes of the brain (for example, DA) (Di Marzo et al., 1998).

The behavioural effects described above may be based on the interaction of endogenous cannabinoids and DA upon gene expression and the accumulation of cyclic adenosine monophosphate (cAMP) in reward-related areas of the brain. SR 141716 has been shown to promote *c-fos* expression in reward-relevant mesocorticolimbic areas by reducing DA D₂ receptor function (Alonso et al., 1999). In addition, DA D₁ receptor activation is critical to ⁹-THC-induced *c-fos* expression in the striatum (Miyamoto et al., 1996). Furthermore, concurrent stimulation of CB₁ receptors and D₂ receptors interact to increase cAMP production, but when either receptor was stimulated alone a decrease in cAMP production was observed (Glass & Felder, 1997).

1.9. Aims of the current thesis

This thesis attempts to further the understanding of the behavioural, neural and emotional effects of cannabinoids. It provides a broad perspective with three major areas of study. These are: 1) the effects of cannabinoids on anxiety-like behaviour (Chapters 2 and 3); 2) the effects of cannabinoids on patterns of brain activation as indicated by *c-fos* expression (Chapter 4); and 3) the addictive potential of CP 55,940

and its capacity to produce sensitization to the effects of cocaine (Chapters 5 and 6). The thesis also addresses the notion that genetic factors may partially determine the behavioural, neural and emotional response to cannabinoids. To this end the “addiction-prone” and “cannabinoid-preferring” Lewis strain of rat is compared to Wistar rats in a wide variety of assays.

It is hypothesised that Lewis rats are less susceptible to the aversive effects of cannabinoids of which anxiety may be a central feature. Assuming that trade-offs occur between reward and anxiety, if Lewis rats are less susceptible to the anxiogenic effects of cannabinoids, then this might provide an explanation for their unique susceptibility to the rewarding effects of such compounds. To provide a test of this assertion, Chapters 2 and 3 compare the effects of the synthetic cannabinoid receptor agonist, CP 55,940 on inbred Lewis rats and Wistar rats in many different animal models of anxiety. These models comprise the social interaction test, the conditioned ultrasonic vocalization (USV) test, the open area avoidance test, the predatory odour avoidance test and the light-dark emergence test.

Chapter 4 assesses whether Lewis rats are uniquely affected by cannabinoids, by assessing the distinct effects that cannabinoids may have on the brain of Lewis rats. This study specifically tests whether CP, 55,940 has distinct effects on the pattern of expression of the immediate early gene, *c-fos*, in the central nervous system (CNS) of Lewis rats in comparison to Wistar rats. It is hypothesised that the effects of CP 55,940 on the pattern or intensity of *c-fos* expression will be different in Lewis rats when compared to Wistar rats.

Chapters 5 and 6 are concerned with cannabinoid-modulation of the neural substrates underlying reward, and the long-term changes that cannabis exposure may evoke in the CNS. Chapter 5 addresses the question of whether behavioural sensitization to the effects of chronic, intermittent exposure to CP 55,940 is able to occur. It is hypothesised that the intermittent administration of CP 55,940 will cause a progressive increase in locomotor activity over days. Further, the study presented in Chapter 5 tests whether the pre-exposure of Lewis rats to CP 55,940 or the co-

administration of CP 55,940 and cocaine, are able to modulate the acute locomotor-activating effects of cocaine and cocaine-induced behavioural sensitization. Thus it is hypothesised that pre-exposure or co-administration of CP 55,940 might increase the locomotor-activating effects of cocaine.

Finally, Chapter 6 extends previous research that has described the rewarding effects of Δ^9 -THC in Lewis rats. Specifically, the effects of CP 55,940 on electrical stimulation of the medial forebrain bundle (MFB) are tested in Lewis rats. It is hypothesised that CP 55,940 will cause a dose-dependent increase in the rewarding efficacy of MFB stimulation. This experiment also tests whether prior CP 55,940 administration causes "rebound dysphoria" or a reduction in the rewarding impact of brain stimulation on the day following cannabinoid exposure. It is hypothesised that prior administration of CP 55,940 may cause a reduction in the rewarding efficacy of MFB stimulation delivered 24 hours later. Furthermore, using the cannabinoid CB₁ receptor antagonist SR 141716, the premise that the endogenous cannabinoid system plays a crucial role in the rewarding effects of brain stimulation is assessed. Thus, it is hypothesised that SR 141716 may dose-dependently reduce the rewarding efficacy of brain stimulation.