CHAPTER 1: INTRODUCTION

1.1 Background to the Research

1.1.1 Cannabis the Plant

The plant termed "*cannabis sativa",* otherwise known as "cannabis", is an annual plant that grows vigorously in sunny environments with fertile soil, and reaches up to five meters in height in a four- to six-month growth cycle (Clarke & Watson, 2002). The search for an active component of cannabis dates back to the nineteenth century, spurred by reports of its extensive medical and hedonistic uses (for review see Mechoulam & Hanus, 2000). However, the major psychoactive constituent in cannabis, THC, was not discovered until over a century later (Gaoni & Mechoulam, 1964). It is now known that cannabis contains more than 60 cannabinoid compounds, as well as hundreds of other constituents (for review see Turner, Elsohly, & Boeren, 1980). Whilst THC is responsible for the psychoactive properties of cannabis, some of the other components modulate its activity (for recent discussion see Di Marzo & Pop, 2004).

Cannabis is mainly used recreationally, and the most commonly used cannabis preparations include "marijuana", "hashish" and "hashish oil". Marijuana is prepared from the dried flowering tops and leaves of the cannabis plant. Hashish or "hash" is composed of compressed cannabis resin, and hashish oil is obtained by using an organic solvent to extract THC from cannabis (Solowij, 1998).

1.1.2 Epidemiology of Cannabis Use

Cannabis is the most widely used illicit substance in Australia (Australian Institute of Health and Welfare, 2005) and the rest of the world

(Rees, Copeland, & Swift, 1998). The onset of recreational cannabis use commonly occurs during adolescence, and sometimes results in decades of daily or near daily use (Scallet, 1991). However, cannabis exposure can also occur earlier in life, as cannabis is one of the most commonly used illicit drugs during pregnancy (Fried & Smith, 2001). The widespread use of cannabis has sparked continuing debates about whether cannabis should be decriminalised for recreational use, and if cannabis should be used therapeutically in medical contexts. These debates have been especially prominent in Australia, where there has been a strong focus on calls for decriminalising recreational cannabis use (Gowing, Ali, Christie, & White, 1998).

1.1.3 Problems in Conceptualising Cannabis Use

In both humans and animals, "acute" and "chronic" cannabinoid effects have been well researched (for review see Solowij, 1998), however, the research on "residual" cannabinoid effects has been limited. It has been argued that the term "residual" is often used to describe effects better characterised as either "acute" or "chronic" effects (Pope, Gruber, & Yurgelun-Todd, 1995). This inconsistent use of terminology has often resulted in ambiguity concerning the specific drug effects under investigation. In the presentation of the current series of terms, "acute effects" refers to those effects produced by a single dose of a cannabinoid lasting for the period of intoxication, and "chronic effects" refers to repeated regular exposure over time (Solowij, 1998). The term "residual effects" refers to the effects of cannabinoids that persist long after the drug has left the central nervous system (CNS) (Pope et al., 1995). "Immature exposure" is defined as cannabinoid exposure occurring at perinatal (Meyer & Kunkle, 1999) or

adolescent ages (Ojeda & Urbanski, 1994), coinciding with major neuronal changes in the CNS. "Mature exposure" is defined as cannabinoid exposure that occurs in the early to mid-adult period of life, when CNS development is largely complete (Luna et al., 2001). Lastly, the term "cannabinoids" refers to compounds that: 1) are structurally related to THC by virtue of a tricyclic chemical structure, 2) possess similar behavioural or physiological effects to cannabis; and 3) bind to cannabinoid receptors (Joy et al., 1999). Therefore, the list is inclusive of THC, synthetic cannabinoids such as CP 55,940, WIN 55,212-2 [4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6H-pyrrolo[3,2,1-i,j]quinolin-6-one], and HU-210 [(-)11-hydroxy-Δ^8-tetrahydrocannabinol-dymethylheptyl]; naturally occurring brain cannabinoids such as anandamide (ANA) and 2-arachidonylglycerol (2-AG) are also classed as cannabinoids (Joy et al., 1999).

1.1.4 Effects of Cannabis

In humans, acute cannabis exposure has been claimed to increase appetite, induce muscle relaxation, sharpen the mind, increase positive mood, relieve anxiety, and have an overall sedative effect (Health Council of the Netherlands: Standing Committee on Medicine, 1996). Additionally, it is believed that cannabis provides relief from symptoms associated with diverse diseases and ailments including chronic pain, spasticity from multiple sclerosis, AIDS (acquired immune deficiency syndrome) wasting, and nausea associated with chemotherapy (Joy et al., 1999). Negative acute effects include anxiety, panic, paranoia, cognitive impairment of attention and memory, psychomotor impairment resulting in increased risk of motor vehicle accident, and an increased risk of developing psychotic symptoms (reviewed

by Hall et al., 1994). Longer term cannabinoid exposure (chronic effects) might include an increased risk of developing cancers of the aerodigestive tract, persistent problems associated with attention and memory, impaired educational attainment and occupational performance, the development of cannabis dependence, and regular symptoms of anxiety, depression, panic, and paranoia (reviewed by Hall et al., 1994). Residual effects include lasting working memory deficits (Schwartz, Gruenewald, Klitzner, & Fedio, 1989), learning impairment (Entin & Goldzung, 1973), attention deficits (Ehrenreich et al., 1999; Solowij, Grenyer, Chesher, & Lewis, 1995), and increased anxiety (Thomas, 1996).

1.1.5 Cannabinoids

There is still much to learn about the mechanisms of cannabinoid action, and more recently it has become apparent that there may be an entire system of endogenous cannabinoids termed "endocannabinoids". The most well known of these, ANA, was discovered in 1992 (Devane et al., 1992). ANA is a fatty-acid derived compound that possesses pharmacological properties similar to THC, which include analgesia, hypothermia, inhibition of locomotor activity (Grotenhermen, 2002) and memory impairment (Mallet & Beninger, 1996). In 1995, a second endocannabinoid called 2-AG was discovered (Mechoulam et al., 1995; Sugiura et al., 1995), and found to have similar pharmacological properties to THC and ANA (Stella, Schweitzer, & Piomelli, 1997). Endocannabinoids are synthesised in neurons, but the process by which they are released is presently unclear. In some instances, they likely diffuse within the membrane, thus activating cells by which they are generated, but there is also evidence that they act on neighbouring cells

(Piomelli, 2003). These endogenous cannabinoids are present only in small amounts in the brain and other tissue (Iversen, 2003).

Inclusive of THC and naturally occurring cannabinoids, synthetic cannabinoids commonly used in scientific research include CP 55,940, WIN 55,212-2, and HU-210. These compounds share similar behavioural and physiological properties to THC, and bind to cannabinoid receptors (for brief review see Joy et al., 1999). Table 1.1 shows some compounds that bind to cannabinoid receptors. In particular, CP 55,940 binds to the brain cannabinoid receptor with high affinity and has been used to label, characterise, and localise cannabinoid receptors in the brain. This binding is not altered by a large range of non-cannabinoid receptor agonists and antagonists, which suggests a high specificity of action (Herkenham et al., 1991).

Table 1.1 Some compounds that bind to cannabinoid receptors

Compound	Properties
THC	Main psychoactive constituent in cannabis
ANA	Primary endogenous cannabinoid agonist in mammals; chemical structure significantly different from plant cannabinoids
2-AG	Endogenous agonist; structurally similar to ANA
CP 55,940	Synthetic cannabinoid; THC analogue; structurally similar to THC
HU-210	THC analogue, 100- to 800-fold greater potency than THC
WIN 55,212-2	Chemical structure different from known cannabinoids, but binds to cannabinoid receptors

Source: Marijuana and Medicine: Assessing the Scientific Base (Joy, Watson, & Benson, 1999).

1.1.6 Cannabinoid Receptors

It was assumed until quite recently that cannabinoids, such as THC, affected neurons by dissolving into lipid components of membranes in a non-specific way. However in the late 1980's, Devane, Dysarz, Johnson, Melvin, and Howlett (1988) discovered specific extracellular receptor proteins for cannabinoids at the surface of neurons. Later, these cannabinoid receptors were divided into two categories, termed cannabinoid receptor 1 (CB_1) and cannabinoid receptor 2 (CB_2). CB_1 receptors are primarily located in the CNS and are thus abundantly expressed in the brain, whereas CB_2 receptors are found in other parts of the body such as the immune system (Ameri, 1999).

Continued research has indicated that CB_1 receptors emerge in the brain during perinatal development, supporting their potential participation in events related to neural development. CB_1 receptor binding and messenger RNA (mRNA) expression in white matter regions have been observed at gestational day 21 (GD 21), PND 1, and PND 5, progressively disappearing by adulthood (Berrendero, Sepe, Ramos, Di Marzo, & Fernández-Ruiz, 1999). However, more recent research indicates that even in the adult brain, CB_1 receptor activity is still occurring, and that both CB_1 receptors and vanilloid type 1 receptors (VR_1) play major roles in regulating neurogenesis in adulthood (Jin et al., 2004).

1.1.7 Brain Mechanisms Underlying Cognition and Emotion

Cannabinoid receptors are implicated in the regulation of memory, learning, and emotion. Herkenham et al. (1990) used receptor autoradiography to localise cannabinoid receptors in the brains of several mammalian species, including humans, and found high densities of CB_1

receptors in the hippocampus, cerebral cortex, cerebellum, and basal ganglia. These receptors are thought to be responsible for cannabinoid effects on memory and cognition (cerebral cortex and hippocampus), motor activity and postural control (basal ganglia, cerebellum), emotion (amygdala and hippocampus), sensory perception (thalamus), and autonomic and endocrine functions (hypothalamus, pons, medulla) (British Medical Association, 1997).

The hippocampus in particular, is thought to be responsible for the learning and memory deficits associated with cannabinoid administration (Deadwyler, Heyser, Michaelis, & Hampson, 1990). Evidence that the hippocampus mediates these cognitive processes was obtained through the direct administration of THC, CP 55,940 and WIN-55,212-2 into the hippocampus of rats, resulting in spatial memory deficits as assessed in an eight-arm radial maze (Lichtman, Dimen, & Martin, 1995).

The amygdala is believed to play a major role in the development and expression of anxiety (Davis, 1992). A study by Onaivi, Chakrabarti, Gwebu, and Chaudhuri (1995) involved the direct injection of THC into the central nucleus of the amygdala of mice, resulting in an instantaneous anxiogenic response. Similarly, acute HU-210 administration has been found to lead to anxiety-like behaviour, and increased *c-fos* activity in the same brain region (Rodríguez de Fonseca, Carrera, Navarro, Koob, & Weiss, 1997).

1.1.8 Age-Dependent Exposure

Rarely have residual cannabinoid effects lasting beyond the drug having left the CNS been investigated, and more specifically, age-dependent exposure has attracted little research interest. This is surprising considering the mounting evidence that cannabis exposure early in CNS development

may lead to permanent changes including alterations in the development of brain transmitter target areas, neurotransmitter system activity, and receptor sensitivity (Mirmiran & Swaab, 1987). The CNS is particularly malleable in early maturation, which arguably renders it especially susceptible to drug-induced neuronal modification (Fernández-Ruiz, Rodríguez de Fonseca, Navarro, & Ramos, 1992). Psychotropic drugs typically interfere with the rigid temporal sequence of events that occur during CNS development (Arenander & de Vellis, 1989). Thus, it has been found that psychoactive drugs can produce long-term alterations when exposure occurs early in life, but only short-term effects when exposure occurs later in life (Mirmiran & Swaab, 1987).

1.1.8.1 Perinatal Exposure

Cannabis is one of the most popularly used illicit drugs during pregnancy (Fried & Smith, 2001). Despite this, the known effects of cannabis exposure on the offspring still remain unclear, largely due to a lack of research. In humans, *in utero* cannabis exposure has been associated with a decrease in birth weight, a possible shortening of gestation, and an increase in the risk of offspring born with birth defects (Hall et al., 1994). In newborns, prenatal exposure to cannabis has been associated with high pitched cries, disturbed sleep, increased tremors, exaggerated startle response, and poor habituation to visual stimuli (Fried & Makin, 1987). Approaching and during school age, perinatal exposure has been associated with deficits in executive function including problems associated with sustained attention, visual perception, language comprehension, and working memory--areas that are associated with the prefrontal region of the brain (Fried, 1995). During

adolescence, perinatal exposure has been linked to poor performance in tasks requiring visual memory, analysis, and integration (Fried, Watkinson, & Gray, 2003).

In rats, perinatal cannabinoid exposure has been associated with changes in the neuroendocrine system such as effects on the reproductive system (i.e., pituitary and gonadal function) in both sexes; during pregnancy, an increase in stillbirths, a decrease in litter size, and lower birth weight; and in the period after birth continued hormonal deviations (for review see Wenger, Croix, Tramu, & Leonardelli, 1992). Further, perinatal cannabinoid exposure can produce both alterations in cannabinoid receptor development, as well as changes in the maturation of several neurotransmitters e.g. dopamine, 5-HT (5-hydroxytryptamine), GABA, and opioid peptides (Fernández-Ruiz et al., 1992; Fernández-Ruiz, Romero, García, García-Palomero, & Ramos, 1997; Fernández-Ruiz, Berrendero, Hernández, Romero, & Ramos, 1999; Fernández-Ruiz, Berrendero, Hernández, & Ramos, 2000). There is also strong evidence of lasting effects on behaviour including, but not limited to, cognitive deficits later in life as demonstrated in tasks assessing anxiety, memory and learning (Gianutsos & Abbatiello, 1972; Kawash, Yeung, & Berg, 1980; Mereu et al., 2003), as well as social and sexual behaviours (Navarro, de Miguel, Rodríguez de Fonseca, Ramos, & Fernández-Ruiz, 1996).

1.1.8.2 Adolescent Exposure

The known effects of cannabis initiation occurring in adolescence remain relatively uncharacterised. This is despite the fact that human cannabis use is commonly initiated in this period of life (Scallet, 1991), and

coincides with major neuronal changes taking place in the CNS (Ehrenreich et al., 1999). Furthermore, in recent years, the age of initiation of cannabis use is becoming earlier in life compared to the recent past. For example, a survey conducted in 1998 found that over 78% of adolescents had reported cannabis initiation at 14 years or younger compared to previous findings of 64% in 1992 (McCreary Centre Society, 1999). It is therefore becoming increasingly clear that conclusive evidence is required to determine whether adolescent cannabis initiation can produce lasting effects on the brain, such as those on cognitive (memory) and emotional (anxiety) processes.

In the rat, major changes in neuronal structure occur at an adolescent age, and the administration of cannabinoids at this time may produce marked changes in neuronal function (Rodríguez de Fonseca, Cebeira, Fernández-Ruiz, Navarro, & Ramos, 1991). A few studies on rats of an adolescent age (30–40 days old) have found residual effects of cannabinoids on working memory (O'Shea et al., 2004), learning (Fehr, Kalant, & LeBlanc, 1976; Fehr, Kalant, & Knox, 1978; Stiglick & Kalant, 1982a; Stiglick & Kalant, 1982b; Stiglick & Kalant, 1983), and anxiety (Ferrari, Ottani, Vivoli, & Giuliani, 1999; Giuliani, Ferrari, & Ottani, 2000; O'Shea et al., 2004). Although few human studies have addressed the lasting neurobehavioural effects of cannabis exposure occurring at adolescent onset, there is emerging evidence that exposure occurring at this time might lead to lasting deficits in attention (Ehrenreich et al., 1999), and working memory (Schwartz et al., 1989).

1.1.8.3 Adult Exposure

Although much of the human research on cannabis exposure is conducted on adult populations, there is a dearth of information on the

consequences of cannabinoid exposure occurring in early adulthood. Some human studies (Ehrenreich et al., 1999; Pope et al., 2003) have examined comparisons of early onset exposure, occurring in the adolescent period versus late onset exposure approaching early adulthood. The results of these studies suggest that there are little to no neurobehavioural deficits associated with late onset exposure. Likewise, the animal literature on adult onset exposure has revealed little evidence of lasting behavioural alterations when performance has been assessed on tasks of learning (Stiglick & Kalant, 1985), and working memory (Deadwyler, Heyser, & Hampson, 1995; Nakamura, da Silva, Concilio, Wilkinson, & Masur, 1991). Indeed, more recent animal studies involving direct adolescent-adult comparisons have shown cognitive deficits specific to adolescent onset exposure (O'Shea et al., 2004; Schneider & Koch, 2003).

1.1.9 Neurological Change Due to Cannabinoid Exposure

In general, the neurological findings on the effects of cannabinoid exposure to date have been mixed. For example, one study (Westlake et al., 1991) involving chronic cannabinoid exposure in rats (THC exposure: 90 days; drug-free: 60 days) and monkeys (cannabis exposure: 1 year; drug-free: 7 months) failed to show cannabinoid receptor alterations (Westlake et al., 1991). Other neurobiological studies however, have shown that cannabinoid exposure can lead to synaptic changes (Scallet et al., 1987), and significant alterations in neurotransmitter systems in the rat brain (Ali et al., 1989). One particular study (Landfield, Cadwallader, & Vinsant, 1988) did not find THC-induced behavioural change, but morphometric analyses revealed alterations in hippocampal structure.

1.1.10 Sex Differences in Cognitive Effects

An emerging theme in the cannabinoid literature is that cannabinoids might exert sex-specific effects. Some studies suggest that cannabis exposure is more psychologically damaging in females. For example, a human study by Patton et al. (2002) found that female cannabis users in particular were susceptible to a fivefold increase in the odds of experiencing depression and anxiety in young adulthood. Yet, it has also been found that these effects in females might be age-specific. For example, adolescent, but not adult onset cannabinoid exposure in female rats has been found to be associated with lasting deficits on cognitive and affective domains (O'Shea et al., 2004). Conversely, some animal studies show that the most pronounced behavioural and neurological change due to cannabinoid exposure occurs in males (for review see Fernández-Ruiz et al., 1992; Moreno, Trigo, Escuredo, Rodríguez de Fonseca, & Navarro, 2003; Navarro et al., 1996). Further, there is evidence that cannabinoids exert sex-specific effects on developing dopaminergic neurons (Fernández-Ruiz et al., 1992). More recently, it was found that THC exposure in the male, but not female foetus, leads to an elevation in the L1 cell adhesion molecule (which plays an important role in cell proliferation and migration, neuritic elongation and guidance, and synaptogenesis), perhaps explaining neurobehavioural disturbances following early life cannabis exposure in males (Gomez et al., 2003).

1.2 Methodological Limitations in Previous Studies

1.2.1 Previous Human Studies

The debatable findings on the residual effects of cannabinoids to date are largely due to methodological issues in human studies. When cannabis

exposure is studied in humans, a number of confounding variables present themselves that complicate, if not obfuscate, the interpretation of research findings. Examples include cannabis dose variations, the use of other drugs, co-existing psychiatric illness, neurological impairment, neurological deficits associated with poor diet leading to nutritional and vitamin deficiencies (Deahl, 1991), and socio-economic variables such as academic or occupational achievement (Pope & Yurgelun-Todd, 1996).

Other methodological problems include small sample size, samples of questionable representativeness, unstandardised psychometric tests, failure to include control groups (Solowij, 1998), a common reliance on self-report questionnaires commonly resulting in inaccurate information, and the use of laboratory-based tasks unrepresentative of everyday functioning and of uncertain clinical relevance (Deahl, 1991). Additionally, a known period of abstinence prior to testing is often difficult to ascertain or is too brief in duration (Pope et al., 1995).

1.2.2 Previous Animal Models

Due to the limitations in human studies, advantages in using animal subjects include the fact that: 1) animals can be bred and maintained under controlled conditions enabling more rigid control of environmental variables and past history, 2) drug purity and dose can be controlled, and 3) a well defined and known period of abstinence can be enforced. This should not be overstated though as animal studies cannot fully substitute for well-controlled human studies, as they do not inform us of the full repertoire of psychological and physiological effects of drugs. Animal studies are nonetheless a

necessary adjunct to human studies because they provide information about how drugs work, which is not obtainable in human studies (Joy et al., 1999).

A common criticism of animal studies is that non-human animals have a very different physiological structure to that of humans; however, animal research continues to confirm that the anatomical structures of both rats and humans are remarkably similar (Joy et al., 1999). This holds true of cannabinoid system physiology as well, supported by the finding that the human cannabinoid receptor (Gerard, Mollereau, Vassart, & Parmentier, 1991) exhibits more than 97 percent identity with the rat cannabinoid receptor (Matsuda, Lolait, Brownstein, Young, & Bonner, 1990). Across species (rat, human, monkey) cannabinoid receptor distribution has been found to be highest in the nuclei of the basal ganglia, the substantia nigra pars reticulata, the globus pallidus, the hippocampus, and cerebellum (areas such as the forebrain and cerebellum are generally implicated in cognition and movement), and lowest in brainstem areas controlling cardiovascular and respiratory functions (Herkenham et al., 1990).

Furthermore, there is strong evidence to suggest that biologically active substances exert their effects similarly at the cellular and molecular level (Joy et al., 1999). As a result of these similarities, the use of animals in biomedical research is firmly entrenched, and there is scarcely a new medicine tested or medical technique tried without prior testing on non-human animals (McDonald & Overmier, 1998).

1.3 Purpose of the Current Research

Previously the author (O'Shea et al., 2004) examined the residual effects of exposure to the synthetic cannabinoid receptor agonist CP 55,940

during either adolescence or early adulthood in female rats. Working memory deficits and decreased social interaction (suggesting increased social anxiety) were observed in adolescent but not adult drug-treated rats several weeks after discontinuation of the drug. These results suggested that chronic exposure to a cannabinoid receptor agonist well after the immediate postnatal period, but before reaching sexual maturity, leads to increased cognitive and affective impairment in later life.

The previous research, however, had some limitations--the residual effects of cannabinoid exposure prior to adolescence were not examined; only female rats were used, ruling out the investigation of possible sex-related differences in cannabinoid effects; and it was not known whether neuronal change accompanied these behavioural deficits.

The current research expands on the previous findings in several ways. First, the current research incorporated perinatal experimental groups. Second, male rats were used to permit comparison with the previous findings on females. Third, the examination of neuronal change was assessed following behavioural assessment. Fourth, to allow for direct behavioural comparisons between the previous and current research, these same paradigms (i.e. object recognition, social interaction) were used, and some additional animal models (double Y-maze, emergence test) were introduced to assess a wider range of behaviours. To achieve these objectives, the current research compares behavioural and neuronal changes in rats when onset of cannabinoid exposure occurs at perinatal (4-day old), adolescent (30-day old), or adult (56-day old) ages. The residual behavioural effects of cannabinoid exposure will be assessed using the double Y-maze (Mallet & Beninger,

1993), the object recognition task (Ennaceur & Delacour, 1988), the social interaction test (File, 1980), and an emergence test (Minor, Dess, Ben-David, & Chang, 1994; Morley, Gallate, Hunt, Mallet, & McGregor, 2001). The double Y-maze task measures learning in a two-component maze comprising of a distinct spatial discrimination (reference memory) and delayed alternation (working memory) component. The object recognition task takes advantage of the innate tendency of rats to explore a novel object more than a familiar object, where a reduced tendency to prefer novel objects more than familiar objects is indicative of working memory dysfunction. The social interaction test allows the analysis of social behaviour between treated rats and age- and treatment-matched conspecifics. Social interaction typically decreases when rats are anxious (File, 1997). Behavioural and pharmacological evidence has suggested that this model is comparable to social anxiety in humans (Kantor, Anheuer, & Bagdy, 2000). The emergence test measures a rat's ethological conflict between exploring a novel environment, and avoiding an open area. This model is considered to be a measure of generalised anxiety since drugs such as diazepam and alprazolam alleviate anxiety in this task (Hascoët & Bourin, 1998).

Previous work conducted in the author's laboratory found altered Fos-immunoreactivity (Fos-IR) in several brain regions of rats exposed to THC during perinatal (Singh, McGregor, & Mallet, In press) and early adult (Singh, McGregor, & Mallet, 2005) developmental periods. These results were thought to provide evidence that brain function was altered in a relatively permanent manner by cannabinoid exposure. However, the methods used in this previous work prevented any clear conclusions from being drawn. That

is, the observed drug-induced alterations in Fos-IR may simply have been the product of a conditioned drug effect (Singh et al., 2005). The present research attempts to settle this matter by examining basal *c-fos* immunohistochemistry in several brain regions of rats exposed to CP 55,940 or its vehicle during perinatal, adolescent and adult ages.

Drug exposure initiated at PND 4 corresponds to the late foetal period in rats and humans. That is, in humans, most synaptic development occurs during the third trimester of pregnancy, but in rats most synaptogenesis occurs during the first few postnatal days (Meyer & Kunkle, 1999). In the rat, the onset of adolescence can be defined as 28-30 days of age (Ojeda & Urbanski, 1994). Adolescence is also a period of major CNS change (Teicher, Andersen, & Hostetter, 1995), and cannabinoid exposure at this time may produce marked changes in neuronal function (Rodríguez de Fonseca et al., 1991). The end of the adolescent period is considered to be between days 38-55 (Ojeda & Urbanski, 1994), therefore 56-day old rats (8 weeks old) in the current study can be considered young adults.

These specific developmental periods were compared as there is growing evidence that the onset of cannabis exposure in the perinatal period can lead to CNS change (Fernández-Ruiz et al., 1992; Fernández-Ruiz et al., 1997; Fernández-Ruiz et al., 1999; Fernández-Ruiz et al., 2000). Likewise, there is mounting evidence that the onset of cannabis exposure at an adolescent age is associated with lasting change in the CNS (Fehr et al., 1976; Fehr et al., 1978; Stiglick & Kalant, 1982a; Stiglick & Kalant, 1982b; Stiglick & Kalant, 1983). It was also important to compare effects in adult

male rats, as previous adolescent, but not adult onset cannabis exposure in females was associated with lasting behavioural change (O'Shea et al., 2004).

1.4 The Cannabinoid CP 55,940

The first experiment (Chapter 3) used THC to assess the effects of perinatal THC exposure on learning. The remainder of experiments (Chapter 4-8) compared the effects of perinatal, adolescent, and adult exposure, using the recently developed THC analogue CP 55,940 as a comparison and alternative to THC. CP 55,940 has behavioural and physiological effects analogous to THC such as analgesia, catalepsy, and hypothermia, which are similar in profile and time-course (Little, Compton, Johnson, Melvin, & Marin, 1988). Further, CP 55,940 has been found to localise and bind to cannabinoid receptors with high affinity (Herkenham et al., 1991), and is consequently a widely used standard agonist in cannabinoid receptor research (Jarvinen, Pate, & Laine, 2002).

Another advantage of using this compound is that THC is sometimes contaminated by naturally occurring microbial toxins, which can themselves expose the human user or animal subject to toxic effects or possible infection (British Medical Association, 1997). Examples of these include *Salmonella muenchen* (Taylor et al., 1982), *Thermactinomyces vulgaris*, *Micropolyspora faeni*, and *Thermactinomyces candidus* (Kurup, Resnick, Kagen, Cohen, & Fink, 1983). Further, cannabis extracts used in the animal literature often have varying concentrations of cannabidiol (CBD) and cannabinol (CBN), which can sometimes increase or decrease the effects of THC, and contamination by other cannabinoids such as cannabigerol and cannabichromene might also affect potency (Stiglick & Kalant, 1983).

1.4.1 Administration and dosage considerations

The main experiments described in this dissertation (Chapter 4-8) employed gradually increasing doses of CP 55,940 to minimise the possibility of drug tolerance. Tolerance is defined here as the reduced effect of a drug due to repeated exposure to the same dose, or the need for progressively larger amounts of the drug to maintain the magnitude of the original effect (Kalant, LeBlanc, & Gibbins, 1971). Gradual increasing doses further mimics human cannabis use, as tolerance to the effects of drugs in humans often results in the user increasing doses of the same drug over time (Hollister, 1998). The use of gradually increasing doses is an important feature of the current research, as immature rats in particular tend to develop tolerance to drugs at a faster rate than mature rats (Barnes & Fried, 1974).

1.5 Methodology

1.5.1 The Double Y-Maze

The double Y-maze task (Mallet & Beninger, 1993) measures both reference and working memory. This maze consists of two Y-shaped mazes joined at the stem (see Figure 1.1). Each trial consists of two components: a *spatial discrimination* in the first 'Y', and a *delayed alternation* in the second 'Y'. The spatial discrimination component is a test of reference (trial independent) memory, while the delayed alternation task is a test of reference memory plus working (trial dependent) memory. Both components make comparable demands on the rat (e.g., motivation, locomotion, sensory-perception of spatial cues) apart from the addition of a working memory task (remembering the location of the food reward from the previous trial) in the delayed alternation component of the task. Therefore, if a rat performs

correctly in the spatial discrimination component of the maze (reference memory), then poorly in the delayed alternation component (reference and working memory), the difference is likely attributed to a failure in working memory (Mallet & Beninger, 1993).

The double Y-maze task has been well validated using pharmacological agents known to enhance memory such as physostigmine, N-methyl-D-aspartate, and FG 7142 (Mason, Mallet, Jhamandas, Boegman, & Beninger, 1999; Smith, Beninger, Mallet, Jhamandas, & Boegman, 1994), and drugs such as muscimol and scopolamine that produce memory deficits (Beninger, Ingles, Mackenzie, Jhamandas, & Boegman, 1992; Beninger, Kühnemann, Ingles, Jhamandas, & Boegman, 1994).

Figure 1.1 The double Y-maze.

1.5.2 The Object Recognition Task

The object recognition task was developed by Ennaceur and Delacour (1988) and takes advantage of the fact that rats investigate novel objects more than familiar objects. See Figure 1.2 for objects used in this task. In this task a reduced ability to discriminate between objects is indicative of working memory dysfunction (Ennaceur, Cavoy, Costa, & Delacour, 1989). The object recognition task (Ennaceur & Delacour, 1988) is a well-validated model of "pure working memory" found to be sensitive to a range of memory-enhancing and memory-impairing treatments. Memory impairment has been produced by scopolamine (Ennaceur & Meliani, 1992). Conversely, object recognition has been enhanced through the administration of drugs such as piracetam and pramiracetam (Ennaceur et al., 1989).

The task consists of two trials with intervening delays (e.g., 2, 6, or 48 h). Preference for the novel object relative to the familiar object typically decreases as delays increase (Ennaceur & Delacour, 1988). In trial 1 (T1), a rat is presented with two identical objects, where it is expected that exploration of the identical objects is similar (see Figure 1.3). In trial 2 (T2), a triplicate of a previously encountered object is presented as well as a new object. In T2 it is expected that "normal" rats recall the previously familiar object and thus attend to the novel object (see Figure 1.4). Object investigation is quantified by measuring the sniffing directed at each object. A recognition index is then calculated by comparing investigation of the novel object relative to the familiar object.

Figure 1.2 Objects used in the object recognition task.

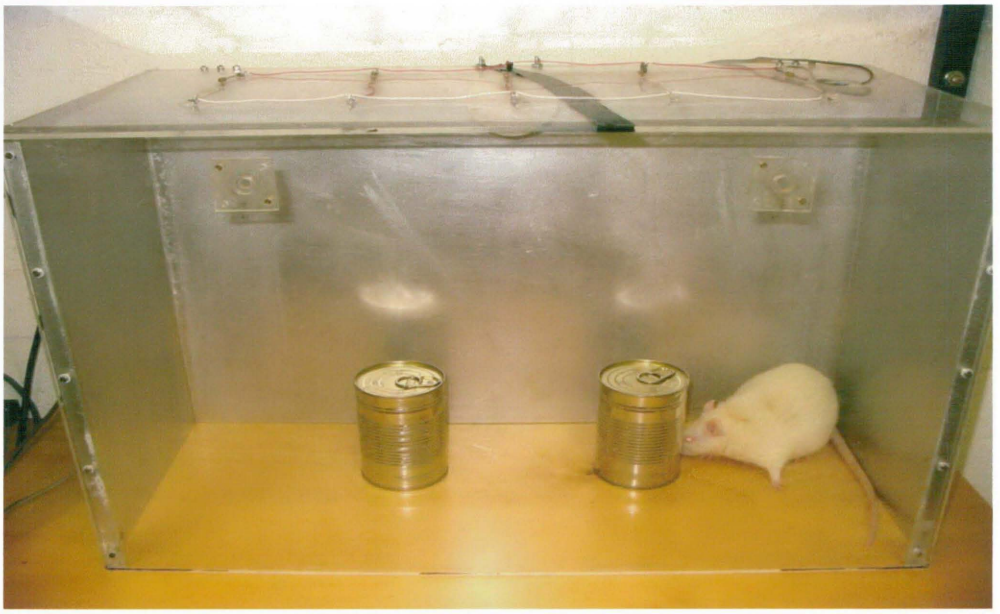

Figure 1.3 T1 in the object recognition task. Two identical objects are presented to the rat.

Figure 1.4 T2 in the object recognition task: a previously encountered object and a novel object are presented to the rat.

1.5.3 The Social Interaction Test

The social interaction test allows the analysis of social behaviour occurring between treated rats and age- and treatment-matched conspecifics. Social interaction typically decreases when rats are anxious. This test also utilises the sensitivity of rat social behaviour to anxiety generated by a novel test arena and/or a brightly lit area (File, 1997). The social interaction test is one of the most extensively used behavioural models of anxiety, and has proven useful in determining the neural basis of anxiety (for review see File & Seth, 2003). The test has been well validated using a variety of anxiolytic (File, Cheeta, & Akanezi, 2001) and anxiogenic (Irvine, Cheeta, Marshall, & File, 2001) drugs, and is useful for screening new pharmacological agents (File, 2000). Anxiogenic drugs such as yohimbine tend to decrease social interaction, whereas anxiolytic drugs such as diazepam (Valium) tend to increase social interaction (for a review of anxiogenic and anxiolytic effects in the social interaction test see File & Seth, 2003). More recently, the social

interaction test has been used to assess the lasting anxiety-like effects of MDMA (3,4-methylenedioxymethamphetamine) (McGregor et al., 2003; Morley et al., 2001) and METH (methamphetamine) treatment (Clemens et al., 2004).

Test arena conditions such as light and familiarity can be manipulated to alter baseline levels of anxiety. Four common conditions are used: 1) low light, familiar arena; 2) low light, unfamiliar arena; 3) high light, familiar arena; and 4) high light, unfamiliar arena. Anxiolytic effects are best detected in conditions that generate low levels of interaction (i.e., high light, unfamiliar arena), whereas anxiety-like behaviour is better detected in the low light, familiar test arena condition (File, 1997). Non-aggressive social behaviours include sniffing, following, grooming, mounting, and crawling under or over the unfamiliar rat (File, 1980). Aggressive behaviours include kicking, jumping on, and wrestling/boxing with an unfamiliar rat (File & Hyde, 1978). See Figure 1.5.

Figure 1.5 The social interaction test.

1.5.4 The Emergence Test

The emergence test (see Minor et al., 1994; Morley et al., 2001), measures a rat's ethological conflict between exploring a novel environment, and avoiding an open area. The basic premise is that the innate tendency to explore a novel place (such as an open area) will be inhibited by the aversive nature of the environment. That is, high levels of exploration in an aversive environment are interpreted as low levels of anxiety-like behaviour (Holmes, 2001). The apparatus consists of a smaller hide box within a larger open field. Various indexes of anxiety are commonly measured in the emergence test. These include emergence latency, emergence frequency, time in the open field, time in the hide box, number of rears, risk assessment (defined as front paws, head and back protruding from the hide box), and transitions between marked squares in the open arena (locomotor activity) (see Figure 1.6).

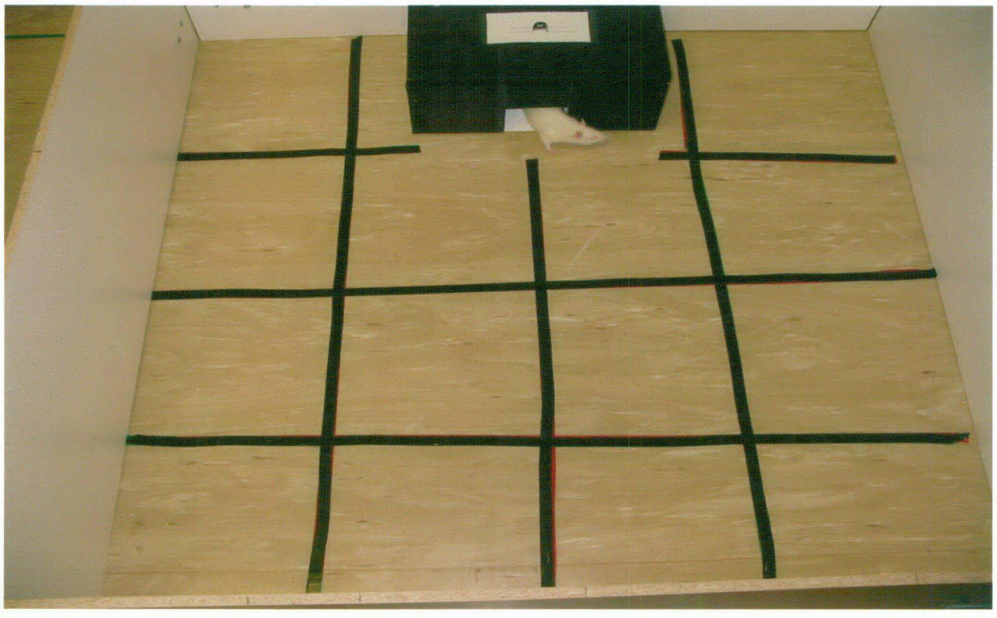

Figure 1.6 The emergence test.

1.5.5 *C-fos* Immunohistochemistry

Over the last decade a number of immediate early genes and associated proteins have been discovered in the brain that are believed to play a role in neuronal plasticity (for review see Hughes & Dragunow, 1995). Immediate early genes such as *c-fos* are rapidly and transiently expressed in response to a variety of stimuli (Harlan & Garcia, 1998), including the induction of stress and the administration of drugs (Hughes & Dragunow, 1995). The immunohistochemical labelling of *c-fos* is considered to be a reliable metabolic marker of neuronal activation (Dragunow & Faull, 1989). Basal levels of *c-fos* expression are low, and become progressively elevated with increasing levels of neuronal metabolic activity (Harlan & Garcia, 1998). A wide range of drugs such as cocaine, morphine, hallucinogens, amphetamines, ethanol, caffeine, nicotine, and THC, have been found to produce clear patterns of *c-fos* expression in distinct areas of the brain (for review see Harlan & Garcia, 1998).

1.6 Hypotheses

In all hypotheses, an age-related effect (i.e., deficits in immature but not mature rats) was not necessarily anticipated as with the author's previous work with female rats (O'Shea et al., 2004), as there is some evidence that male rats incur greater long-lasting cannabinoid-induced change (for review see Fernández-Ruiz et al., 1992; Moreno et al., 2003; Navarro et al., 1996).

Hypothesis 1:

In the double Y-maze, it was predicted that both the perinatal THC- and vehicle-treated groups would acquire the spatial discrimination (reference memory) component of the maze at similar rates. In the delayed alternation

(working memory) component of the maze, it was predicted that THC-treated rats would acquire learning at a slower rate than the vehicle-treated controls. The dissociation between components for THC-treated rats was predicted to confirm that any observed differences were due to a specific deficit in working memory rather than an effect on reference memory.

Hypothesis 2:

In the object recognition task, no differences in exploration of the identical objects were expected in T1 for all groups. In T2, it was expected that the immature CP 55,940-treated groups (i.e., the perinatal and adolescent drug-exposed groups) would show a decreased ability to discriminate between novel and familiar objects relative to immature controls. In adult groups, it was also anticipated that the CP 55,940 group could manifest a decrease in the ability to discriminate between novel and familiar objects relative to controls. In terms of locomotor activity, no differences between cannabinoid and vehicle groups were anticipated.

Hypothesis 3:

In the social interaction test, it was expected that the immature CP 55,940 groups would display significantly decreased social interaction (an anxiogenic effect) relative to immature controls. Evidence of this would manifest in terms of decreases in rat social behaviours such as sniffing, following, grooming, mounting, and crawling under or over an unfamiliar conspecific. In adult groups, it was also anticipated that the CP 55,940 group could show a decrease in social interaction relative to adult controls.

Hypothesis 4:

In the analysis of aggressive behaviours in the social interaction test (kicking, jumping on, wrestling/boxing with the unfamiliar rat), it was anticipated that immature CP 55,940 groups would show increased aggressive behaviours in a social interaction test relative to immature controls. In adult groups, it was also anticipated that the CP 55,940 group could exhibit greater aggressive behaviour relative to adult controls.

Hypothesis 5:

In the emergence test, it was expected that the immature CP 55,940 groups would primarily show an increase in time spent in the hide box (anxiety-like behaviour) relative to immature controls. Other behaviours examined such as emergence latency, emergence frequency, duration of time in the open field, number of rears, and risk assessment (front paws, head and back protruding from the hide box) were also predicted to act as indicators of anxiety-like behaviour. In adult groups, it was also anticipated that the CP 55,940 group would show greater anxiety-like behaviour relative to adult controls.

Hypothesis 6:

In using *c-fos* immunohistochemistry to detect differences in neuronal activation between drug-treated and vehicle groups, *c-fos*-immunoreactive cells were quantified in specific brain regions and subregions. It was predicted that the immature drug-treated groups would exhibit altered *c-fos*-immunoreactivity relative to the immature controls due to drug-induced neuronal change produced by CP 55,940 during development. In adult rats, it was anticipated that significant differences in *c-fos* expression might also be

exhibited between the CP 55,940- and vehicle-treated groups irrespective of mature age at exposure.

1.7 Experimental Design

The THC study (i.e., double Y-maze) from start to finish spanned close to 3 months. Perinatal rats were administered THC from PND 4-14, followed by a 42 day drug-free period, prior to 25 days of maze acquisition.

CP 55,940 experiments spanned approximately 5 months per developmental group. Due to the time demands associated with these studies, each developmental group underwent this sequence at separate points in time. That is, perinatal groups underwent 21-days of drug administration, a 28-day drug-free period, testing in the object recognition task (6 days), testing in the social interaction test 2 days later (1 day testing), testing in an emergence test 2 days after social interaction testing (1 day testing), and finally immunohistochemistry 2 days after this. This histological procedure from start (perfusions) to finish (quantifying mounted brain slices) spanned approximately 1 month. Scoring behavioural tests, data consolidation, and statistical analysis spanned approximately 2 months. Following this, adolescent rats were assessed, followed by adult rats.

1.8 Organisation of Dissertation

Chapter 1 presents the background to the research, methodological limitations in previous studies, purpose of the current research, the cannabinoid CP 55,940, methodology, hypotheses, experimental design, and organisation of dissertation. Chapter 2 presents a detailed review of the literature on the neurobehavioural effects of cannabinoids in both humans and animals. Chapter 3 examines the effects of perinatal THC exposure on

learning in a double Y-maze task. In Chapter 4, the effects of perinatal, adolescent, and early adult CP 55,940 exposure on working memory are examined using an object recognition task. In Chapter 5, the effects of perinatal, adolescent, and adult CP 55,940 exposure on social anxiety in later adulthood are examined using a social interaction test. In Chapter 6, aggressive social behaviours were examined at all ages. In Chapter 7, the effects of perinatal, adolescent, and early adult CP 55,940 exposure on generalised anxiety are examined in an emergence test. In Chapter 8, drug-induced alterations of neuronal activity in these same rats are assessed using *c-fos* immunohistochemistry. Finally, Chapter 9 presents a general discussion.

CHAPTER 2: A REVIEW OF THE FINDINGS ON THE NEUROBEHAVIOURAL EFFECTS OF CANNABINOIDS

2.1 Introduction

Scientific research on cannabis dates back as far as the 1840s (for review see Mechoulam & Hanus, 2000). Schlesinger (1840) was thought to be the first investigator to obtain an active extract from the leaves and flowers of hemp. Over a hundred years later, Decourtive (1948) described the preparation of an ethanol extract that upon evaporation of the solvent gave a dark resin, which he termed "cannabin". Research on cannabis has grown rapidly since, but little is known about the lasting (residual) effects of cannabis on the CNS (Pope et al., 1995). In particular, the effects of cannabinoid initiation at key developmental ages (i.e., perinatal, adolescent, and young adult periods) have received sparse interest. To date, behavioural studies of animals (Gianutsos & Abbatiello, 1972; Kawash et al., 1980; Mereu et al., 2003) and humans (Fried et al., 2003; Richardson, Ryan, Willford, Day, & Goldschmidt, 2002) suggest that perinatal cannabinoid exposure is associated with cognitive deficits and neurological change (Fernández-Ruiz et al., 1992; Fernández-Ruiz et al., 1997; Fernández-Ruiz et al., 1999; Fernández-Ruiz et al., 2000). The animal literature on adolescent onset cannabis exposure suggests that adolescent (Fehr et al., 1976; Fehr et al., 1978; Stiglick & Kalant, 1982a; Stiglick & Kalant, 1982b; Stiglick & Kalant, 1983), but not adult (Stiglick & Kalant, 1985) cannabinoid exposure onset is associated with cognitive deficits in later life. In humans, early but not late adolescent onset exposure has been associated with lasting cognitive deficits (Ehrenreich et al., 1999; Pope et al., 2003), and changes in brain morphology have been

observed in early adolescent users (Wilson et al., 2000). Further, recent animal studies involving direct adolescent-adult comparisons have shown cognitive deficits specific to adolescent onset exposure (O'Shea et al., 2004; Schneider & Koch, 2003). A review of the literature on the residual effects of cannabinoid exposure on animals was conducted over a decade ago (Scallet, 1991). This review supported these age-related findings, and suggested that the lasting neurotoxic effects of THC appear to be specific to young rats (40-days old or less), when exposure is chronic (90 days+; 8-10% of a rats life span). The current review of the literature considers both animal and human research on perinatal, adolescent, and adult onset cannabis exposure.

2.2.1 Perinatal Human Studies

Very little research has been conducted on the effects of *in utero* cannabis exposure in humans. However, one longitudinal study examining the long-term consequences of *in utero* cannabis exposure is "The Ottawa Prenatal Prospective Study" which began in 1978 (Fried et al., 2003). This study has followed a group of at-risk individuals (males and females) from birth to adolescence (Fried & Smith, 2001). So far, the results suggest that *in utero* cannabis exposure is negatively associated with tasks requiring visual memory, analysis, and integration. Fried (1985) noted some potential confounds in this study--pregnant cannabis using women differed from the rest of the sample in terms of socioeconomic and education level, and the use of other drugs (such as tobacco and alcohol) prevented direct inferences of cannabis effects.

Another longitudinal study termed the "Maternal Health Practices and Child Development Project" (Leech, Richardson, Goldschmidt, & Day, 1999)

assessed 6-year olds perinatally exposed to illicit substances. Pregnant women were light to moderate users of alcohol and cannabis whose use decreased after the first trimester of pregnancy. Tobacco was used by the majority of women and did not change during pregnancy. Second trimester cannabis use was associated with increased impulsivity and positive effects on attention (n.b. cannabis exposed children made fewer errors of omission, therefore, had a lower rate of missing the stimulus). A follow-up study (Richardson et al., 2002) assessed offspring at 10 years of age. Perinatal cannabis exposure was found to be associated with learning and memory deficits, as well as impulsivity. Some methodological limitations of these studies (Leech et al., 1999; Richardson et al., 2002) were communicated by the authors: most pregnant women recruited were of lower socioeconomic status, and tobacco and alcohol use was also quite high in cannabis-using women.

By and large, in humans, ethical considerations limit the evaluation of the effects of perinatal cannabis exposure to clinical observations and longitudinal studies. Of particular concern, these studies are typically correlational in nature, and therefore cannot establish a causal relationship between cannabis use and subsequent neurocognitive alterations (for review see Abel, 1985).

2.2.2 Perinatal Animal Studies

Due to the limitations in human studies, animal models are regularly used to establish causal links between drug exposure and neurobehavioural change. Perinatal exposure to a cannabis extract in male and female rats has been associated with learning deficits (Lashley III maze) in adult life

(Gianutsos & Abbatiello, 1972). Cannabis-treated rats required more trials to reach an acquisition criterion, committed more errors, and spent more time completing acquisition trials. A subsequent study (Kawash et al., 1980) found learning deficits (Morris water maze) in young male and female rats (PND 22) treated with a cannabis extract during the perinatal period. Cannabis-treated rats required a significantly greater number of trials to master the maze than did controls.

Effects on physical development and motor behaviours have also been observed following perinatal THC administration (Borgen, Davis, & Pace, 1973). For example, during the first postnatal days, THC-exposed rats showed delayed incisor eruption, cliff avoidance, and visual placing reflexes. At PND 9, these rats exhibited hyperactivity in an open field, and reduced rearing and grooming at PND 13 and 17. Similarly, perinatal ANA exposure has been associated with impaired responsiveness to a challenge with ANA or THC in later life i.e. PND 40 (Fride & Mechoulam, 1996), as demonstrated by a lack of immobility in the ring test for catalepsy, hypothermia, and analgesia. Without challenge, these rats exhibited a spontaneous decrease in open field activity, catalepsy, hypothermia, and a hypoalgetic tendency.

Sex-differences in cannabinoid effects are an emerging theme in the current literature. In adulthood, female rats treated with THC in perinatal life (Navarro, Rubio, & Rodríguez de Fonseca, 1994) have exhibited increased rearing and locomotor activity in familiar environments. In novel environments, these same females exhibited lower locomotor activity in a sociosexual approach test, and increased emergence latency (anxiety-like behaviour) in the light-dark emergence test. Additionally, THC-exposed

females exhibited elevated plasma corticosterone in comparison to THC-treated males. This study suggests that the greatest neurobehavioural effects of cannabinoid exposure are demonstrated in females. However, these same investigators (Navarro et al., 1996) showed pronounced behavioural deficits in male rats treated with THC at perinatal ages. In adulthood, male THC-treated rats exhibited marked changes in sociosexual behaviour compared to THC-treated females and controls. In the light-dark emergence test, performance between THC-treated rats and controls was comparable, yet THC-treated males exhibited reduced anxiety in a social interaction test. The behavioural alterations observed in THC-exposed males were paralleled by a significant decrease in L-3,4-dihydroxyphenylacetic acid contents in the limbic forebrain as measured by high performance liquid chromatography (HPLC). Another study (Moreno et al., 2003) showed that perinatal THC exposure in males in particular, resulted in a significant increase in immobility, decreased locomotion, and an altered behavioural response to dopaminergic receptor agonists.

As illustrated by another study (Rubio et al., 1995), these sex-related differences in perinatal cannabinoid effects do not follow a predictable pattern. Both male and female THC-treated rats showed increased rearing, grooming, and sniffing, in adulthood. THC-exposed males exhibited increased exploratory behaviour in a plus-maze paradigm. Further, female THC-treated rats showed greater locomotor activity than controls, and exhibited higher levels of both corticotropin releasing factor (CRF-41) in the medial basal hypothalamus (MBH) and plasma corticosterone. THC-exposed males showed the lowest levels of both endocrine parameters.

In males, perinatal exposure to WIN 55,212-2 (Mereu et al., 2003) has been found to disrupt glutamate release in the hippocampus and long-term potentiation (LTP). More recently (Singh et al., In press) adult male rats exposed to THC in perinatal life have exhibited altered basal levels of *c-fos* expression in the core and shell of the nucleus accumbens (NAC), islands of Calleja (ICjM), bed nucleus of the stria terminalis (BNST), central nucleus of the amygdala (CEA), dorsolateral and lateral periaqueductal grey (PAG), ventral tegmental area (VTA), and Edinger-Westphal nucleus (EW).

To date the literature suggests that perinatal cannabinoid exposure is associated with a range of neurobehavioural changes across sex and species. For a summary of both perinatal human and animal studies see Table 2.1.

2.2.3 Adolescent Human Studies

The terms "puberty" and "adolescence" are sometimes used interchangeably, however puberty largely refers to the acquisition of mature reproductive function, whereas adolescence refers to the maturation of cognitive and social behaviours (Sisk & Foster, 2004). A key feature of adolescence is that both sexual development and brain maturation take place concurrently (see Sisk, Schulz, & Zehr, 2003). The effects of cannabis initiation occurring in adolescence remain relatively uncharacterised. This is despite the fact that human cannabis use is commonly initiated in this period of life (Scallet, 1991).

Human studies suggest that cannabis exposure during adolescence can lead to lasting memory deficits. For example, one study (Schwartz et al., 1989) found short-term memory deficits (test battery included the Wechsler Intelligence Scale for Children-WISC) in adolescent cannabis users following

6 weeks of abstinence. Adolescent onset cannabis exposure is also associated with deficits specific to attention, particularly in male users of the drug (Pope & Yurgelun-Todd, 1996). Further, cannabis use in females is correlated with visuospatial memory deficits (Pope, Jacobs, Mialet, Yurgelun-Todd, & Gruber, 1997). However, a shortcoming of this study (Pope & Yurgelun-Todd, 1996; Pope et al., 1997), and many others, is the use of a brief drug-free period prior to testing (i.e., only 1 day abstinence or at least 19 h). Thus, it is likely that the effects of continued drug residue in the CNS and withdrawal effects confounded these results.

A similar study (Fried, Watkinson, James, & Gray, 2002) found that current but not former heavy cannabis use in adolescence was associated with declines in IQ (as measured by WISC in childhood and the Wechsler Adult Intelligence Scale-WAIS in later years). However, the authors noted that the IQ declines among heavy current users likely reflected the effects of drug residue.

An emerging pattern in the cannabinoid literature is that neurobehavioural deficits are specific to early adolescent exposure. For example, a study by Ehrenreich et al. (1999) found that early (before age 16) but not late (16 years old+) onset cannabis exposure is associated with attention deficits specific to visual scanning. However, the drug-free period was too brief in duration (i.e., M=28.8 h prior to testing, ranging from 2 h to 1 week). The authors acknowledged that these results likely represented drug residue as opposed to lasting cannabis effects.

Pope et al. (2003) conducted a similar study comparing early (before age 17) versus late (17 years old+) onset cannabis exposure. In this

Table 2.1 A brief overview of the lasting neurobehavioural effects of perinatal cannabis exposure onset in both humans and animals.

Investigators	Drug	Species	Age	Drug Admin Age	Drug-free	Test Age	Sex	Behavioural and/or Neurological test	Result
Fried et al. (2003)	Cannabis moderate: <6 joints p.w.; heavy: >6 joints p.w.)	Human	Gestation	1st -3rd trimester	Varied	Birth-adol	M\F	Tests of general intelligence, achievement, executive functioning (i.e., problem solving, focused attention, working memory), & inhibition of proponent & self-directed responses	Deficits in visual memory, analysis, & integration
Leech et al. (1999)	Cannabis (2+ joints per month during 1st trimester)	Human	Gestation	1st- 3rd trimester	6 years	6 years old	M\F	Continuous Performance Test, Stanford-Binet Intelligence Scale	2nd trimester: increased impulsivity & positive effect on attention
Richardson et al. (2002)					10 years	10 years old		Wisconsin Card Sorting Test, Wide Range Assessment of Memory & Learning, Trail Making Test, The Stroop Test, Grooved Pegboard Test, & Continuous Performance Test	Impulsivity, learning & memory deficits
Gianutsos and Abbatiello (1972)	Cannabis extract (250 THC mg/kg)	Rat	GD 8	GD 8-11	75 days*	PND 65	M	Lashley III maze	Deficits in learning
Borgen et al. (1973)	THC (10 mg/kg)	Rat	GD 10	GD 10-12	18 days*	PND 9-21	M	Physical maturation & reflexive behaviour	Delayed incisor eruption, cliff avoidance, & visual placing
								Open field test	Hyperactivity, decreased rearing & grooming
Kawash et al. (1980)	Cannabis extract (4.2 THC mg/kg)	Rat	GD 2	GD 2-6	37 days*	PND 22	M\F	Morris water maze	Deficits in learning
Navarro et al. (1994)	THC (5mg/kg)	Rat	GD 5	GD 5- PND 24	46 days*	PND 70	F	Locomotor activity	Familiar environment- increased rearing & locomotion
							F	Sociosexual approach test	Novel environment- lower locomotor activity
							M\F		Increased grooming
							F	Light-dark emergence test	Anxiety-like behaviour
							F	Radioimmunoassay	Increased plasma corticosterone

Investigators	Drug	Species	Age	Drug Admin Age	Drug-free	Test Age	Sex	Behavioural and/or Neurological test	Result
Rubio et al. (1995)	THC (5 mg/kg)	Rat	GD 5	GD 5- PND 24	46 days*	PND 70	M\F	Motor Behaviours	Increases in rearing, grooming, & sniffing
							F		Increased locomotor activity
							M	Plus maze	Increased exploratory activity
							M\F	Plasma corticosterone analysis	In females, higher CRF-41 in MBH & plasma corticosterone
Navarro et al. (1996)	THC (20 mg/kg)	Rat	GD 5	GD 5- PND 24	46 days*	PND 70	M	Sociosexual approach test	Opposite pattern to controls
							M	Light-dark emergence test	No change
							M	Social interaction	Anxiolytic behaviour
							M	HPLC	In limbic forebrain, decreased L-3,4-dihydroxyphenylacetic acid
							F	All tests	No neurobehavioural deficits
Fride and Mechoulam (1996)	ANA (20 mg/kg)	Rat	GD 14	GD 14-18	25 days*	PND 22-40	M\F	Open field test	Decreased locomotion
								Ring test for catalepsy, hypothermia, & analgesia	Impaired response to ANA or THC- lack of immobility. Without challenge- decrease in open field activity, catalepsy, hypothermia, & hypoalgetic tendency
Mereu et al. (2003)	WIN 55,212-2 (0.5 mg/kg)	Rat	GD 5	GD 5-20	41 & 81 days	PND 40& 80	M	Passive avoidance task	Memory deficits
						PND 40		LTP	Disruption to hippocampal LTP
						PND 40& 80		Microdialysis	Disruption of glutamate release
Moreno et al. (2003)	THC (0.1, 0.5, 2 mg/kg)	Rat	GD 5	GD 5- PND 24	46 days*	PND 70	M\F	Open field test: 1) spontaneous behaviour, 2) behavioural response to apomorphine, & 3) behavioural response to quinpirole	Males: increase in immobility, decreased locomotion, & altered response to dopaminergic agonists
Singh et al. (In press)	THC (5 mg/kg)	Rat	PND 4	PND 4-14	42 days*	PND 56	M	*C-fos* immunohistochemistry	Increased Fos-IR in NAC (core and shell), ICjM, BNST, CEA, PAG (dorsolateral & lateral), VTA, & EW

*Drug-free estimates based on gestation period of 21 days e.g. drug admin GD 8-12 & testing on PND 65 is calculated 21-12=9 + PND 65= 74 days drug-free. If last drug admin occurred on a PND e.g. GD5 to PND 24 & testing at PND 70, this was calculated as PND 70-24= 46 days drug-free.

instance, a 28-day period of controlled abstinence was initiated prior to testing. The results indicated that early onset users exhibited poorer overall IQ than late onset users. Further analyses controlling for Verbal IQ scores were conducted, and these differences were no longer significant. The authors identified several possible confounds in this study including unidentified variables such as the use of other drugs, neuropsychological deficits, and psychiatric disorders. Further, cannabis users likely differ from the mainstream population due to their drug-using activities, which many in turn affect opportunities for academic, social, and practical learning (Pope et al., 2003).

Despite these issues, it is interesting to note that the findings of both these studies (Ehrenreich et al., 1999; Pope et al., 2003) coincide with evidence that early onset cannabis exposure is associated with altered brain function and morphology. For example, Wilson et al. (2000) used magnetic resonance imaging (MRI) and positron emission tomography (PET) to assess early (before age 17) versus late onset (17+) cannabis exposure in adolescence. Early onset users had smaller brains on the whole, a reduced percentage of cortical gray matter, and a larger percentage of white matter volume. Further, early onset use in males was associated with significantly higher cerebral blood flow (CBF).

2.2.4 Adolescent Animal Studies

The findings on adolescent cannabinoid exposure in animals largely mirror human findings. A few studies on rats of adolescent age have examined the residual effects of cannabinoids on learning (Fehr et al., 1976; Fehr et al., 1978; Stiglick & Kalant, 1982a, 1983). In these studies, varying

doses of THC (10-20 mg/kg) were administered to 30-40 day old male rats for 1-6 months, followed by a drug-free period lasting 1-2 months. Impairments on radial arm maze learning and motor coordinated tasks were observed in rats treated with high doses for 6 months. In males, these behavioural impairments were accompanied by irregular spike-like activity in the hippocampus as measured by electroencephalogram (EEG) (Fehr et al., 1978). Another study (Stiglick & Kalant, 1982b) used a drug-free period lasting 2-3 months and found that rats treated with THC (20 mg/kg) for 3 or 6 months showed deficits in DRL (differential reinforcement of low-rate responding) and locomotor activity. Further, shuttle-box avoidance learning was facilitated in THC-treated rats following a 4- to 6-month drug-free period (Stiglick, Llewellyn, & Kalant, 1984).

In contrast, some studies show a lack of association between adolescent onset cannabis exposure and neurobehavioural change. For example, Slikker et al. (1992) trained 64 peri-adolescent male rhesus monkeys (2-3 years old) to perform several complex behavioural tasks prior to the initiation of 1 year of chronic cannabis exposure. Deficits in motivation to respond to behavioural tasks were evident during treatment, but not observed after discontinued exposure. Additionally, neurohistological and electronmicroscopic measures failed to detect neurochemical alteration 7 months after exposure.

Other studies (Ferrari et al., 1999; Giuliani et al., 2000) have found that the administration of HU-210 in adolescent male rats is associated with persistent learning deficits and anxiety-like behaviour. However, as both

these studies involved a drug-free period of only 7 days, these findings were not sufficient to determine whether these residual deficits were permanent.

Some studies have involved direct adolescent-adult comparisons. For example, a study by Schneider and Koch (2003) exposed adolescent (40-day old) and adult (70-day old) male rats to WIN 55,212-2 over 25 days, followed by a 10 day drug-free period. Long-lasting deficits in sensorimotor gating, object recognition and performance in a progressive ratio task were apparent in adult rats exposed to WIN 55,212-2 in the adolescent, but not adult period of life. Moreover, these authors (Schneider & Koch, 2004) found that both perinatal medial prefrontal cortex (mPFC) damage alone, and combined with subsequent adolescent WIN 55,212-2 exposure leads to deficits in social play, social behaviour unrelated to play, and self-grooming in later life. These data suggest that the mPFC likely plays an important role in social behaviour and anxiety. A similar study conducted by the author (O'Shea et al., 2004) compared the effects of CP 55,940 in adolescent (30-day old) and adult (56-day old) female rats. Poorer working memory and decreased social interaction (increased social anxiety) was observed in adolescent CP 55,940-treated rats.

The direct adolescent-adult comparisons provide new evidence that cannabinoid-induced behavioural deficits might be specific to adolescent exposure. However, longer drug-free periods beyond those used in these studies i.e. 10 (Schneider & Koch, 2003) and 21 days (O'Shea et al., 2004), are required to determine whether these residual cannabinoid effects are permanent. For a summary of both adolescent human and animal studies see Table 2.2.

Table 2.2 A brief overview of the lasting neurobehavioural effects of adolescent cannabis exposure onset in both humans and animals.

Investigators	Drug	Species	Age	Drug Admin	Drug-free	Test Age	Sex	Behavioural and/or Neurological test	Result
Schwartz et al. (1989)	Cannabis (18 g p.w. average)	Human	14-16 years old	4 months+ (mean 7.6 months)	6 weeks	Adol varied	M\F	WISC; tests measuring auditory/ verbal, visual/spatial, immediate & delayed short-term memory; & construction ability	Deficits in short-term memory
Pope and Yurgelun-Todd (1996) Pope et al. (1997)	Cannabis heavy users: 29/30 days; light users: 1/30 days)	Human	18-28 years old	2 years+	19-24 h	Adol-adult	M\F	Test battery: intelligence, abstraction ability, sustained attention, verbal fluency, & learning Additional attention subtests: Continuous Choice Reaction Time, Stroop Task, Letter Detection Task, & Checkerboard Test	Impairments in attention/executive functioning: greatest effects in males No differences between heavy and light users. Deficits in visuospatial memory in female heavy users
Ehrenreich et al. (1999)	Cannabis 1 day p.w. for 6 months	Human	<16 or 16+	6 months+	29.8 h average	Adol varied	M\F	Test battery: divided attention, working memory, mental alertness, mental flexibility, & visual scanning	Early onset use: attention deficits specific to visual scanning
Wilson et al. (2002)	Cannabis	Human	Early (<17) vs. late onset (17+)	Varied	Varied	Young adult	M\F	MRI & PET	Early onset use: changes in brain morphology. In males, increased CBF
Fried et al. (2002)	Heavy cannabis users: >5 joints p.w.; light users: <5 joints p.w.; & former users	Human	17-20 years old	Not specified	Current use-3 months	Adol-young adult	M\F	WISC: at pre-adolescent period (9-12 years old) WAIS: young adult (17-20 years old)	Current heavy use associated with declines in global IQ, but no declines in IQ for former users
Pope et al. (2003)	Heavy cannabis users: >5000 times; former users: <5000	Human	Early (before age 17) or late (17+) onset user	1-5000 times	28 days	30-55 years old	M\F	Neuropsychological tests measuring attention, verbal & visuospatial memory, & executive functions	Early onset use: poorer Verbal IQ, but further analyses revealed that differences no longer significant
Fehr et al. (1976)	THC (10-20 mg/kg)	Rat	30-day old	1-6 months	25 days-2 months+	Adult	M	Hebb-Williams closed field maze	Learning & motor deficits in rats treated with the high dose for 6 months
Fehr et al. (1978)	THC (20 mg/kg) THC (10 mg/kg)	Rat Rat	30-day old GD 8 (perinatal)	6 months GD 8-12	1-2 months 74 days*	Adult PND 65	M M\F	Moving belt test Muricidal behaviour EEG Hebb-Williams maze	Learning impairments Increased muricidal behaviour Irregular spike-like activity in hippocampus No learning deficits

Investigators	Drug	Species	Age	Drug Admin	Drug-free	Test Age	Sex	Behavioural and/or Neurological test	Result
Stiglick and Kalant (1982a)	THC (20 mg/kg)	Rat	40-day old	3 or 6 months	1 month	Adult	M	Eight-arm maze	Learning impairments in rats treated from 3-6 months
				3 months				12-arm maze	Learning deficits in rats treated for 3 months
Stiglick and Kalant (1982b)				3 or 6 months	2-3 months			Open field activity	Increased locomotor activity in rats treated for 3 or 6 months
								DRL	Impairments in DRL performance
Stiglick et al. (1984)				3 months	4-6 months			Shuttle-box avoidance	Avoidance learning facilitated 4-6 months after drug-administration
Stiglick and Kalant (1983)	THC (20 mg/kg)	Rat	40-day old	3 months	1-4 months	Adult	M	12-arm radial maze	Low performance
								DRL	Low performance
								Two-way shuttle-box	Learning slightly facilitated
								Open field	Initial hypoactivity→ hyperactivity but not significant
Slikker et al. (1992)	Cannabis: 1) high dose, 2) low dose, 3) sham ethanol & 4) sham smoke	Monkey	2-3 years old	1 year	<2 months	Adol	M	Operant behavioural tasks	No deficits in motivation
					7 months			Neurohistological & electromicroscopic measures	No neurochemical alteration
Ferrari et al. (1999)	HU-210 (0.025-0.1 mg/kg)	Rat	230-250 g	4 days	7 days	Early adult	M	Morris water maze	Disruptions in spatial acquisition, & vocalisation at high dose
Giuliani et al. (2000)	HU-210 (0.025-0.1 mg/kg)	Rat	200-230 g	9 days	7 days	Early adult	M	X-maze	Anxiety-like behaviour at highest dose
Schneider and Koch (2003)	WIN 55,212-2 (1.2 mg/kg)	Rat	40-day old (adol) & 70-day old (adult)	25 days	10 days	Adult	M	Object recognition task, progressive ratio, operant behaviour task, locomotor activity, & prepulse inhibition of acoustic startle response	Deficits in sensorimotor gating, object recognition, and performance in a progressive ratio task in adolescent rats
O'Shea et al. (2004)	CP 55,940 (0.15-0.30 mg/kg)	Rat	Adol (30-day old) & adult (56-day old)	21 days	21 days	Adult	F	Object recognition task Social interaction test	Deficits in object recognition, & increased social anxiety in adolescent rats
Schneider and Koch (2004)	WIN 55,212-2 (1.2 mg/kg)	Rat	40-day old lesioned animals	25 days	15 days	80-day old	M	Play behaviour, social behaviour, & self-grooming	Deficits in play behaviour, social behaviour, & self-grooming

2.2.5 Adult Human Studies

Although the bulk of cannabinoid research is conducted on adult populations, there is little information on the lasting effects of cannabinoid exposure onset at this age. However, a highly publicised human longitudinal study found lasting cognitive deficits in former users of the drug (Soueif, 1971; Soueif, 1975, 1976), as shown by poor performance on various neurological tests. These results were reported as residual effects on cognitive processes thought to be due to CNS alteration. However, tainting this claim were variables such as varying rates of literacy and educational attainment between groups, previous opiate and alcohol use, significant age differences between the hashish and control groups, diverse durations of previous hashish use between groups, and a variable period of abstinence prior to testing (Fletcher & Satz, 1977).

Adult cannabis exposure has also been associated with deficits specific to attention. For example, a case study by Solowij et al. (1995) was conducted to determine whether known selective attention deficits are reversed following cessation of cannabis use. The participant in this study was a 35-year old male with an 18-year history of cannabis use. This individual was reported to have a minimal history of other drug use, underwent psychological assessment throughout testing and at follow-up, and was required to provide daily urine samples to verify the absence of cannabis. Selective attention was assessed at 1, 3, and 6 weeks post-cessation as measured by brain event-related potential (ERP). The ERP patterns (reflecting difficulties in filtering out irrelevant stimuli) suggested that no recovery of selective attention was shown following 6 weeks cessation. The

authors noted that caution needs to be exercised in interpreting the results of a single case study, but an interesting additional finding was that a single acute dose of cannabis reversed these attention deficits.

More recently, adult onset cannabis use was associated with deficits in memory and other executive functions. A sample of cannabis users were assessed after 28 days abstinence (Bolla, Brown, Eldreth, Tate, & Cadet, 2002). These individuals had smoked cannabis for a minimum of 2 years, but were users of other drugs such as alcohol (less than 14 alcoholic drinks per week), caffeine, and tobacco. Nonetheless, they were excluded from the study if found to be dependent on alcohol or other psychoactive substances. Heavy cannabis use was associated with persistent decrements in neurocognitive performance (i.e., on tests measuring memory, executive functioning, psychomotor speed, and manual dexterity). The authors (Bolla et al., 2002) noted that this study was limited by small sample size, self-report questionnaires, and a failure to include a control group of non-users.

2.2.6 Adult Animal Studies

In animals, the research evidence largely suggests that adult onset cannabinoid exposure does not result in cognitive change. Stiglick and Kalant (1985) were interested in determining whether age-related effects might explain their previous findings of learning deficits following cannabinoid exposure in immature rats (Fehr et al., 1976; Fehr et al., 1978; Stiglick & Kalant, 1982a; Stiglick & Kalant, 1982b; Stiglick & Kalant, 1983). As a comparison, THC (20 mg/kg) was administered to 70-day old adult male rats for 3 months. Following a 1- to 4-month drug-free period, no performance deficits were observed on a eight-arm radial maze task, in a DRL task, or

open field activity, however, two-way shuttle box avoidance learning was facilitated by previous cannabis treatment. The authors concluded that immature rats are more vulnerable to lasting cannabinoid effects than mature rats.

Likewise, there is little evidence that adult onset cannabinoid exposure produces memory deficits. Deadwyler et al. (1995) assessed adult rats (approximately 70-days old) 15 days after a 90-day THC administration protocol. No evidence of deficits in short-term memory in a DMTS (delayed-match-to-sample) task was shown. Another study (Nakamura et al., 1991) found no evidence of memory deficits in 56-day old rats following a 30-day drug-free period, despite chronic exposure of up to 90 days. These findings agree with others (O'Shea et al., 2004; Schneider & Koch, 2003), but some research does show a link between adult onset exposure and lasting brain change (Scallet et al., 1987).

For example, Scallet et al. (1987) used light and electron microscopy to quantify the effects of chronic THC exposure on the anatomical structure of the hippocampus. Adult (140 days old) rats were administered THC for 90 days. Following a 7 month drug-free period, the ultrastructural appearance of neurons was altered and the volume of neurons and their nuclei in the CA3 region was reduced. Further, a 44% reduction in the number of synapses per unit volume was found. Golgi impregnation studies on additional THC-treated rats also showed a reduction in the dendritic length of CA3 pyramidal neurons, despite no apparent changes in hippocampal ultrastructure, and no changes in synaptic density. It was concluded that the observed hippocampal changes

might constitute a morphological basis for behavioural effects following chronic exposure to THC.

Another study (Landfield et al., 1988) assessed behavioural performance in adult (84-112 days old) male rats up to 2 weeks after 8 months of THC treatment. THC-treated rats showed little behavioural change (as assessed in a open field test, active avoidance task, and maze reversal tasks), but morphometric analyses showed significant THC-induced changes in hippocampal structure; specifically, THC-treated animals exhibited decreased neuronal density and increased glial cell reactivity (i.e., an increase of cytoplasmic inclusions).

Some studies show opposite neurological findings. For example, Ali et al. (1989) investigated whether THC produces alterations in various neurotransmitter systems (i.e., dopamine, serotonin, acetylcholine, gamma amino butyric acid (GABAergic), benzodiazepine, and opiate). Adult rats (56-70 days old) were administered THC for 90 days. Two months after the cessation of THC exposure, a significant decrease in GABA receptor binding in the hippocampus of animals in the high dose group was observed. This study was replicated, but no evidence of hippocampal change was found. Consequently, the investigators concluded that chronic exposure to THC does not alter neurotransmitter systems.

Likewise, other studies have found no evidence of altered brain morphology by cannabinoid exposure. For example (Westlake et al., 1991), cannabinoid receptors in the striatum, cerebral cortex, cerebellum, hippocampus, and brainstem/spinal cord were not irreversibly altered by chronic-THC exposure in adult (56-70-day old) rats, despite a long drug-

administration (90 days) and drug-free period (60 days). In addition, peri-adolescent male monkeys were treated with cannabis for a year, followed by 7 months abstinence. Cannabinoid receptors in the caudate, prefrontal cortex, and cerebellum of the monkey brain were not irreversibly altered by chronic cannabis exposure.

On the contrary, a recent study conducted in the author's laboratory (Singh et al., 2005) using *c-fos* immunohistochemistry, involved the administration of THC (0.05-5 mg/kg) to adult male rats (56-day old). Following a substantial drug-free period (55 days), THC-treated rats displayed increased Fos-IR in the medial caudate putamen (CPU), NAC (core and shell regions), lateral septum (LS), CEA, and PAG (lateral). These results were thought to provide evidence that brain function continues to be altered weeks after cannabinoid exposure has ceased. However, as with previous work (Singh et al., In press), the authors cautioned that the alteration in Fos-IR observed might have been due to a motivational state previously associated with drug administration. For a summary of both human and animal adult studies see Table 2.3.

Table 2.3 A brief overview of the lasting neurobehavioural effects of adult cannabis exposure onset in both humans and animals.

Investigators	Drug	Species	Age	Drug Admin	Drug-free	Test Age	Sex	Behavioural and/or Neurological test	Result
Soueif (1971, 1975, 1976)	Cannabis (0.21-1.08 g) per day	Human	Adol-adult (15-50+ years old)	5-30 years	Varied	Adult	M	Perceptual speed & accuracy, distance & time estimation, immediate memory, visual-motor abilities, & reaction time	Poor performance on neurological tests in cannabis group
Solowij et al. (1995)	Cannabis (6 g) per day	Human	Adult (35 years old)	18 years	6 weeks	Adult	M	ERP	Deficits in selective attention
Bolla et al. (2002)	Cannabis (3 times p.w.+)	Human	Adult (18-37 years old)	2 years+	28 days	Adult	M\F	Test battery: intelligence, language skills, verbal & visual memory, attention & concentration, executive functioning, visuoperception/visuoconstruction, psychomotor speed, & manual dexterity	Heavy use associated with deficits in neurocognitive performance (i.e., memory, executive functioning, psychomotor speed, & manual dexterity)
Stiglick and Kalant (1985)	THC (20 mg/kg)	Rat	70-day old	3 months	1-4 months	Adult	M	Eight-arm radial maze, DRL task, Open field activity Shuttle box avoidance learning	No deficits Avoidance learning facilitated
Scallet et al. (1987)	THC (20-60 mg/kg)	Rat	140-day old	90 days	7 months	Adult	M	Light & electron microscopy	Change in hippocampal appearance & decreased mean volume of neurons
	THC (10-20 mg/kg)	Rat		90 days	2 months	Adult		Golgi impregnation studies	A reduction in neuron dendrite length
Landfield et al. (1988)	THC (4-10 mg/kg)	Rat	84-112 days old	4-8 months	Varied	Adult	M	Open field test, Active avoidance task, Maze reversal task	Few behavioural differences in all tests
					<2 weeks			Light & electron microscopy	Cytoplasmic inclusions in hippocampus
Ali et al. (1989)	THC (0-20 mg/kg)	Rat	56-70 days old	90 days	24 h, 2 months	Adult	M	Neurotransmitter concentration & receptor binding	2 months drug-free: decreases in GABA receptor binding in hippocampus for high dose group
	THC (0-60 mg/kg)	Rat		90 days	2 months	Adult	M		No decrease in GABA receptor binding in hippocampus
Nakamura et al. (1991)	THC (5 mg/kg)	Rat	56-day old	90 days	30 days	Adult	M	Eight-arm radial maze	No memory deficits
Westlake et al. (1991)	THC (0-60 mg/kg)	Rat	56-70 days old	90 days	60 days	Adult	M	Cannabinoid receptor binding	Cannabinoid receptors not altered
	Cannabis (2.6% THC)	Monkey	3.7±0.5 kg	1 year	7 months	Adol	M	Cannabinoid receptor binding	Cannabinoid receptors not altered
Deadwyler et al. (1995)	THC (10 mg/kg)	Rat	Adult (70-day old)	35 days	15 days	Adult	M	DMTS task	No memory deficits
Singh et al. (2005)	THC (0.05-5 mg/kg)	Rat	Adult (56-day old)	41 days	55 days	Adult		*C-fos* immunohistochemistry	Increased Fos-IR in medial CPU, NAC (core & shell), LS, CEA, & PAG (lateral)

2.3 Discussion

The literature review of research on human perinatal cannabis exposure suggests that *in utero* exposure commonly spans from 1st to 3rd trimester pregnancy. These longitudinal studies have involved both male and female offspring of females who had used cannabis during pregnancy, and assessment to date has ranged from birth to a young adult age. So far, the findings of these studies suggest that cannabis exposure is associated with a range of subtle, but detectable, cognitive deficits in later life.

Perinatal cannabinoid studies in animals have generally involved a drug administration period ranging from 3 to 40 days, and a drug-free period spanning 18 to 81 days. Most of these studies have involved both male and female offspring of cannabinoid treated dams, and like the findings on human cannabis exposure, almost all of these studies found evidence that perinatal cannabinoid exposure leads to neurobehavioural deficits in later life. Interestingly, two of these studies (Moreno et al., 2003; Navarro et al., 1996) found that males exhibit greater behavioural deficits than females. Taken together, the perinatal findings across species suggests that exposure at this age can result in lasting neurobehavioural change in both sexes.

The human research on adolescent onset cannabis exposure generally involved a drug administration period ranging from as little as 1 day to more than 2 years. The drug-free period varied from 2 h to 3 months. The bulk of these studies involved both male and female participants, although the proportion of male participants was significantly higher in most instances. The overall results of these studies suggest that adolescent onset cannabis exposure is associated with lasting cognitive deficits. Two of these studies

(Ehrenreich et al., 1999; Pope et al., 2003) compared early versus late adolescent onset cannabis exposure, and found cognitive deficits specific to early exposure. One study found pronounced cognitive deficits in males (Pope & Yurgelun-Todd, 1996), whilst another found greater deficits in females (Pope et al., 1997).

The animal research on cannabinoid exposure during adolescence has involved drug administration ranging from as little as 4 days to 6 months. Drug-free periods varied from 7 days to 6 months. The majority of these studies have only investigated lasting cannabinoid effects in male rats, and suggest that adolescent onset cannabinoid exposure leads to lasting neurobehavioural change. In contrast, studies on adolescent monkeys failed to show cannabinoid receptors change (Westlake et al., 1991) and overall brain morphology (Slikker et al., 1992), up to 7 months after a 1 year drug-administration period. By and large, the research outcomes following adolescent onset cannabinoid exposure largely mimic the perinatal findings.

Human research on cannabis exposure at an adult age has involved participants who have used cannabis for up to 30 years. The period of abstinence form cannabis use ranges from current use to up to 6 weeks drug-free. Most of these studies have involved males, and the findings of these studies suggest that both current and former cannabis use likely leads to subtle cognitive deficits.

Cannabinoid research on adult animals has generally involved drug administration from 35 days to 1 year. As with many other studies, the drug-free period varies (i.e., from 24 h to 7 months). These studies have primarily used male rats, and just over half of these studies suggest that adult onset

cannabis exposure is associated with drug-induced neuronal change, particularly hippocampal alterations.

On the whole, these studies suggest that immature organisms are more vulnerable to cannabinoid-induced neurobehavioural change than mature organisms. These findings concur with a previous review (Scallet, 1991), suggesting that lasting cannabinoid effects are specific to immature rats (40-days old or less). These effects are likely attributable to the fact that the CNS is particularly malleable in early maturation, which arguably renders it susceptible to drug-induced neuronal modification (Fernández-Ruiz et al., 1992). Psychotropic drugs have been found to interfere with the rigid temporal sequence of events that occur during CNS development (Arenander & de Vellis, 1989), and consequently produce long-term alterations when exposure occurs early in life, but only short-term effects when exposure takes place at a later age (Mirmiran & Swaab, 1987).

Having said this, neurobehavioural deficits sometimes accompany adult onset cannabinoid exposure. Thus, these cannabinoid-induced deficits in adulthood contradict with age-related findings of greater vulnerability at a younger age. However, these conflicting findings might be partly attributable to sex-related differences in cannabinoid effects. Some animal studies show that the most pronounced behavioural and neurological change due to cannabinoid exposure is found in males (for review see Fernández-Ruiz et al., 1992; Moreno et al., 2003; Navarro et al., 1996). Further, there is evidence that these profound sex-related behavioural effects in male rats might be mediated by dopaminergic neurons (Fernández-Ruiz et al., 1992).

Further, age- and sex-specific effects might be paradigm-dependent. For example, evidence of sex-specific effects has been observed in animal models (i.e., the holeboard, the open field, and the elevated plus-maze). The nature of these effects depended on the sex of the animal and the specific model used (Biscaia et al., 2003). Furthermore, age-related effects have been shown in paradigms such as the light-dark test (Hascoët, Colombel, & Bourin, 1999). For example, male mice of varying ages were compared and it was found that the youngest and oldest animals exhibited least fear (Hascoët et al., 1999). The authors suggested that these differences might be related to deficits in brain maturity for younger animals, and functional deficits in older animals.

In sum, the current review suggests that cannabinoid exposure throughout life may compromise neurobehavioural function. These findings also point to possible sex differences, which warrant further study. However, these findings are not sufficient to determine whether these deficits are permanent. Nonetheless, there is growing evidence that cannabis exposure, spanning from the perinatal period through to adulthood, might lead to lasting and potentially irreversible cognitive and physiological change.