FOREWORD TO CHAPTER 3

Chapter 3 is based on an article published in the journal *Behavioural Pharmacology* devoted to the cannabinoids. This article appears in volume 16, issues 5-6, pages 455-461, 2005. Paul E. Mallet, my primary PhD supervisor appears as the co-author on this paper, which is titled "Impaired learning in adulthood following neonatal Δ^9-THC exposure". This research was supported by an Australian Research Council (ARC) Discovery Grant and a University of New England Research Assistantship (UNERA).

CHAPTER 3: IMPAIRED LEARNING IN ADULTHOOD FOLLOWING NEONATAL Δ^9-THC EXPOSURE

Abstract

Cannabis is one of the most commonly used illicit drugs during pregnancy, but little is known about the lasting effects of early life exposure to this drug. In this study, twelve male Wistar rat pups were treated daily with THC (5 mg/kg s.c.) or its vehicle between PND 4 and 14. Drug administration during this early postnatal period in rats is analogous to the third trimester of gestation in humans, which is a major period of synaptogenesis. Rats were subsequently tested drug-free during young adulthood (PND 56) using a two-component food-motivated double Y-maze test. Each trial included distinct *spatial discrimination* and *delayed alternation* components, which permitted the simultaneous assessment of reference memory and working memory. Rats were tested for 30 trials per day, 5 days per week for 5 weeks. Results revealed no significant differences between THC- and vehicle-treated rats in the spatial discrimination task. However, compared to vehicle-treated rats, THC-treated rats committed significantly more errors, and required significantly longer to obtain 80% correct performance over 2 consecutive days in the delayed alternation task. Results suggest that neonatal THC exposure leads to a specific and lasting deficit in learning in adulthood, which is likely due to impaired working memory function.

3.1 Introduction

The ancient use of cannabis in obstetrics and gynecology is well documented (Russo, 2002a). Pregnant women commonly used cannabis based preparations to reduce nausea and to induce and facilitate childbirth (Russo, 2002a). Today, cannabis is one of the most widely used illicit drugs during pregnancy (Fried & Smith, 2001). Although some pregnant women continue to use the drug for medicinal purposes (Russo, Dreher, & Mathre, 2002b; Westfall, 2004), most modern usage can be characterised as recreational. Human and animal research shows that cannabinoids readily cross the placental barrier (Blackard & Tennes, 1984; Hutchings, Martin, Gamagaris, Miller, & Fico, 1989), and can be transferred to the infant through breast milk (Jakubovic, Hattori, & McGeer, 1973). Little, however, is known regarding the adverse cognitive effects of perinatal exposure that persist into adulthood.

Recent studies suggest that long-term gestational cannabis exposure can lead to increased impulsivity (Leech et al., 1999), learning and memory deficits (Richardson et al., 2002), and difficulties in visual memory, analysis and integration (Fried et al., 2003). However, these correlational studies cannot establish a causal relationship between cannabis use and subsequent neurocognitive alterations. Thus, there is a need for laboratory studies using animal models to uncover causal evidence concerning the long-lasting effects of perinatal cannabis exposure on cognitive function.

Few animal studies have examined the consequences of perinatal cannabinoid exposure on cognitive function. Of these, it has generally been noted that *in utero* exposure to cannabis extracts results in maze learning

impairments in the exposed offspring later in life (Gianutsos & Abbatiello, 1972). A recent study using rats (Mereu et al., 2003) found that *in utero* exposure to the synthetic cannabinoid receptor agonist WIN 55,212-2 disrupted retention of a passive avoidance task in 40- and 80-day old offspring, suggesting that memory was permanently impaired by cannabinoid exposure during a critical developmental period. However, it is noteworthy that these studies alone do not provide definitive evidence that perinatal cannabinoid exposure impairs cognitive processes that influence memory. In some instances, behavioural deficits may have been the result of impaired attention, reduced motivation, or deficits in perceptual or motor abilities. Furthermore, animals are typically exposed to the drug *in utero* by injecting the pregnant dams. In humans, substantial synaptic development occurs during the third trimester of pregnancy, but in rats most synaptogenesis occurs during the first few postnatal days (Meyer & Kunkle, 1999). The administration of cannabinoids to pregnant rats may therefore bypass the most critical period of development likely to inflict permanent changes in cognitive function.

To overcome some of these limitations, the present study examined the effects of perinatal exposure to THC in laboratory rats on acquisition of a double Y-maze task. The double Y-maze task consists of separate spatial discrimination and delayed alternation components, and includes control procedures that guard against misinterpretation of possible non-mnemonic effects such as altered motor function, motivation, or sensory-perceptual abilities (Mallet & Beninger, 1993; Mason et al., 1999; Smith et al., 1994). The delayed alternation task, but not the spatial discrimination task, requires the

use of working memory. A selective impairment in the delayed alternation task can therefore be confidently attributed to a deficit of working memory.

Rat pups were treated with THC or its vehicle from PND 4 and 14. This developmental period is analogous to the late foetal period in humans when most synaptogenesis occurs (Meyer & Kunkle, 1999). Maze testing took place in early adulthood (i.e., beginning on PND 56). It was predicted that THC would impair memory, which would be evidenced by delayed acquisition of the delayed alternation component but not the spatial discrimination component of the double Y-maze task.

3.2 Materials and Method

3.2.1 Subjects

At PND 2, 48 albino Wistar rat pups were pooled, sexed, and randomised into six groups of six males and two females each. Each group then was allocated randomly to one of the postpartum dams. Pups were rubbed into the home cage bedding prior to introduction to the foster mother to mask previous scents. At all times rats had free access to food and water and were housed in a temperature- and humidity-controlled colony room (22 ± 2 °C) on a 12-h light/12-h dark cycle. On PND 21 pups were weaned and female pups were culled.

Of these 48 rats, 12 male rats were used for the behavioural testing described below, which commenced on PND 56. Five of these rats comprised the THC-exposed group and the remaining seven rats served as vehicle controls. Each rat was weighed on PND 52 and 53 and the mean of these values was used to determine a baseline weight. Rats were then maintained at 85% of their individual free-feeding weights throughout the testing period by

rationing the amount of food provided each day. Target weights were adjusted by 10 g per week to allow for normal growth.

3.2.2 Drug Preparation and Administration

Δ^9-Tetrahydrocannabinol (98% purity) was purchased from the Australian Government Analytical Laboratories (Pymble, NSW, Australia) as an ethanol solution. The solution was mixed with a small amount of Tween 80 (polyoxyethylene sorbitan monoolate, ICN Biochemicals, Seven Hills, NSW, Australia), and then stirred under a stream of nitrogen gas until the ethanol was evaporated. Saline (0.9%) was then added such that the final solution contained 1 mg/ml THC in a vehicle consisting of 15 µl Tween 80 per ml saline. THC and vehicle solutions were administered subcutaneously (s.c.) once per day from PND 4 to PND 14 in a volume of 5 ml/kg body weight, yielding a dose of 5 mg/kg. Body weights were logged during the drug administration period.

3.2.3 Apparatus and Procedure

A double Y-maze (Figure 3.1) was used. The maze walls (26 cm high x 1 cm thick) were constructed of white melamine. Removable white melamine doors (26 cm high) were used to restrict access to each half of the maze when required. Each arm of the maze (35 cm long) extended from the central stem (45 cm long x 17 cm wide) at a 120° angle. The entire maze was supported on a table 70 cm above floor level in a room where a variety of visual cues (e.g., posters, light fixture, door, experimenter) were readily observable by the rat. The table acted as a floor to the maze, which was cleaned between trials (1:10 white vinegar:water). Small pieces of Froot Loops cereal (Kellogg's) were used as rewards, and were available via food

cups (4 cm wide x 1 cm deep) placed in the central stem and arms of the maze. A pink-noise generator (K2135 Personal Noise Generator, Altronics, Perth, Western Australia) provided low-level ambient masking noise in the experimental room.

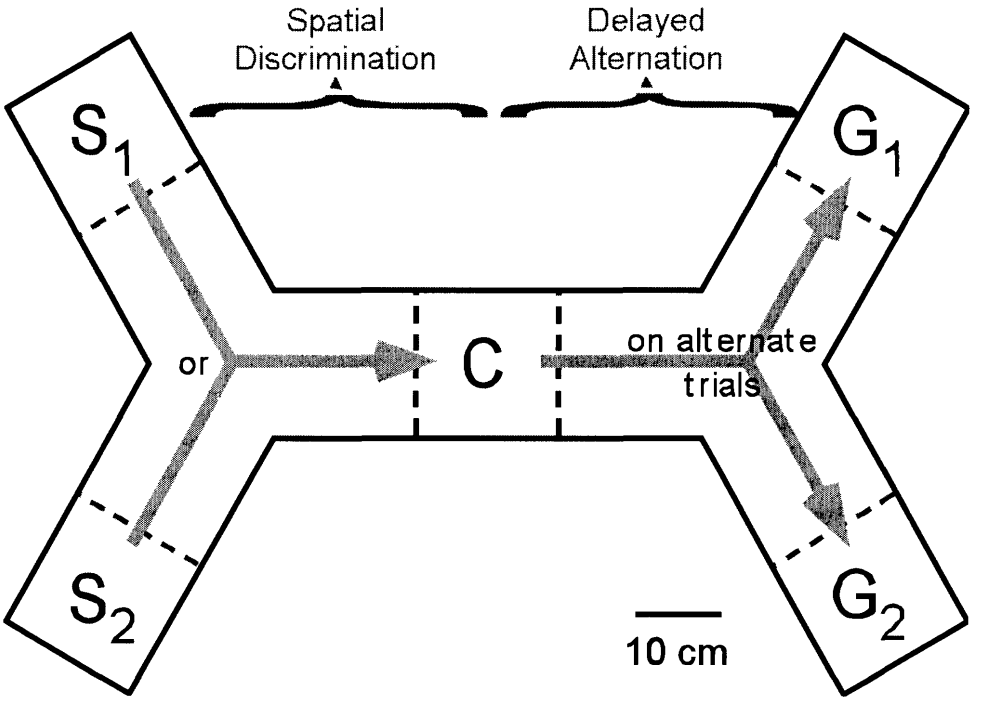

Figure 3.1 Schematic representation of a top view of the double Y-maze. Broken lines indicate the location of removable guillotine doors that were used to restrict or allow access to maze arms. Each trial began by quasi-random placement in either start box S_1 or S_2. Following a correct entry and subsequent reward in the central box C, rats were given access to the second portion of the maze where food reward was available in either goal box G_1 or G_2, with the correct arm alternating on successive trials. Each trial therefore comprised a spatial discrimination, followed by a delayed alternation. Arrows represent possible correct paths to obtain food reward. Rats received 30 consecutive trials, 5 days per week, for 5 weeks.

3.2.3.1 Habituation

Rats were familiarised with the maze during three consecutive daily sessions, beginning on PND 56. During each 15-min habituation session rats had free access to all parts of the maze to collect cereal pieces from food

cups. Beginning the second session maze doors were gradually inserted and removed until rats showed no overt signs of fear.

3.2.3.2 Testing

Testing occurred 5 days per week (Monday-Friday) over the following 5 weeks. Beginning on PND 59, each rat received one session comprising 30 trials per day. Each trial comprised two distinct components: a *spatial discrimination* in the first 'Y' followed by a *delayed alternation* in the second 'Y'. The spatial discrimination component is a test of reference (i.e., trial-independent) memory, while the delayed alternation component is a test of reference memory plus working (i.e., trial-dependent) memory. Both components require a two-arm choice in a Y-maze, and therefore have similar task demands in terms of motivation, locomotion, and sensory-perceptual abilities. However, the delayed alternation task requires the rat to remember the location of the food reward in the previous trial. If a treatment selectively affects choice accuracy in the delayed alternation component of the maze, one can confidently attribute the effect to an alteration of cognitive abilities that influence working memory (Mallet & Beninger, 1993).

First, each rat was placed into either start box 'S_1' or 'S_2' of the first 'Y' with the door at the distal end of the centre box 'C' closed (see Figure 3.1). The start box door was opened, and the correct action was to enter box 'C' without first entering the other start box. If the rat entered the incorrect arm (i.e., the other start box), no reward was available but no intervention took place. Instead, the rat was permitted to locate the reward in box 'C'. Once the rat had collected the reward in box 'C', a door was placed behind the rat

(at the proximal end of 'C') to prevent retreat back into the first 'Y'. This constituted the end of the spatial discrimination component of each trial.

The distal door of box 'C' was then removed, and the rat was presented with the second 'Y'. This time the animal's task was to choose alternate arms on successive trials, irrespective of the original start arm. In other words, if the reward was placed in 'G_1' on the first trial, it was now placed in 'G_2' on the second trial, and so on (Figure 3.1). For the first trial of each session, the initial goal box ('G_1' or 'G_2') was randomly allocated. After a correct choice, the rat received its cereal reward, and then was returned immediately to 'S_1' or 'S_2' to begin the next trial. The procedure was identical following an incorrect choice, with the exception that no reward was provided. This constituted the end of the delayed alternation component of the task. The rat was removed and the next trial began immediately afterwards. Approximately 4 sec was required between trials to return the rat to the start box, re-bait the food cups, and position the doors. Rats typically required 2-3 sec to complete the spatial discrimination task, thus the inter-trial interval between successive spatial alternation trials was approximately 7 sec.

The first trial of each session comprised an additional un-scored trial where doors were used to block the incorrect arms. Start boxes ('S_1' or 'S_2') were chosen randomly for each trial, with the provisos that half the trials per session began in each start box, and neither start box was used for more than three consecutive trials. The initial goal box also was randomly allocated, with the proviso that an equal number of each goal box was allocated each day. Rats were single caged for approximately 1.5 h within a few hours of the

completion of each session for feeding, and were then returned to their group cages where they remained until the next test session.

3.3. Statistical Analysis

The number of correct entries out of 30 trials in both the spatial discrimination and delayed alternation components were converted to a percentage for each rat for each of the 25 test sessions. Two-way (treatment by day) analysis of variance (ANOVA) with repeated measured on the 'day' factor were conducted separately for the spatial discrimination and delayed alternation components. Where a significant treatment by day interaction was observed, Bonferroni-adjusted unpaired *t*-tests were used to compare vehicle- and THC-treated groups at each level of day (α=0.002).

As a secondary measure of maze performance, the number of days required to reach 80% correct performance over 2 consecutive days was calculated for each animal. The vehicle- and THC-treated groups were compared using separate unpaired *t*-tests for the spatial discrimination and delayed alternation components. Those rats that did not obtain at least 80% correct over 2 consecutive days over the course of the 25 test days were given the maximum score of 25 days.

Perinatal body weights, daily weight gained, and adult body weights at PND 52 and 53, were analysed using two-way (treatment by day) mixed-design ANOVAs with repeated measures on the day factor. All ANOVAs and *t*-tests were conducted using SPSS 12.0.1 for Windows.

In some instances the ANOVA sphericity assumption was violated. When this occurred, the Huynh-Feldt epsilon correction was applied, but this

did not alter the outcome of any of the analyses. For ease of interpretation only the uncorrected ANOVA results have been presented.

3.4 Results

3.4.1 Spatial Discrimination

Choice accuracy in the spatial discrimination task was highly accurate for both the vehicle- and THC-treated animals (Figure 3.2, A). All rats were performing near the ceiling within the first few days of testing and by day 3 performance in the spatial discrimination was error-free for all rats for the remainder of the sessions. The two-way ANOVA revealed a significant main effect of day [$F(24,240)=32.18$, $p<0.001$] and treatment by day interaction [$F(24,240)=2.26$, $p<0.01$]. The main effect of treatment [$F(1,10)=2.13$, $p>0.05$] was not significant. The mean ± standard error of the mean (SEM) number of days required to obtain 80% correct performance over 2 consecutive days was 2.60 (± 0.24) and 2.71 (± 0.18) for the THC and vehicle groups, respectively. The difference between the two groups was not significant [$t(10)<1.0$]. (For statistics outputs see Appendix A1 & A2 and for data see Appendix B1).

3.4.2 Delayed Alternation

In general, choice accuracy gradually improved in both groups over days. The number of correct choices in the delayed alternation component was similar in both groups during the first 16 days. From that point forward the number of correct choices was higher in the vehicle group compared to the THC group, but the difference between groups narrowed towards the completion of testing (Figure 3.2, B). This description of the data was supported by statistical analysis. The two-way ANOVA revealed a significant

main effect of treatment [F(1,10)=9.68, $p<0.05$], a significant main effect of day [F(24,240)=16.03, $p<0.001$], and a significant treatment by day interaction [F(24,240)=6.78, $p<0.001$]. Bonferroni-adjusted *t*-tests comparing groups at each level of day revealed that the THC group made fewer correct choices than the vehicle group on day 17 [$t(10)=5.94$, $p<0.001$], 19 [t(10)=4.83, $p<0.01$], 20 [$t(10)=5.41$, $p<0.001$], 21 [$t(10)=6.29$, $p<0.001$], and 22 [$t(10)=5.18$, $p<0.001$]. The number of days required to obtain 80% correct performance over 2 consecutive days also differed between groups. The mean (± SEM) was 24.60 (± 0.40) and 17.57 (± 0.95) for the THC and vehicle groups, respectively. This group difference was significant [$t(10)=5.94$, $p<0.001$]. (For statistics outputs see Appendix A3 & A4 and for data see Appendix B2).

3.4.3 Body Weight

Body weight increased at a rapid rate in both groups throughout the perinatal drug treatment period (PND 4-14). At the beginning of the drug treatment period body weights were similar in both groups, but growth in the THC-treated group fell slightly behind that of the vehicle-treated rats over days (Figure 3.3, A). This appeared to be due to reduced daily growth in the THC group towards the middle of the drug treatment period (Figure 3.3, B). The two-way ANOVA conducted on the perinatal body weight data revealed a significant main effect of drug treatment [F(1,46)=4.76, $p<0.05$], a significant main effect of day [F(10,460)=608.54, $p<0.001$], and a significant treatment by day interaction [F(10,460)=2.05, $p<0.05$]. The two-way ANOVA conducted on the daily weight gain data revealed a significant main effect of treatment

[F(1,46)=5.60, $p<0.05$], but the main effect of day and the treatment by day interaction were not significant [F(9,414)<1.0].

In adulthood, body weights in the THC group were slightly lower than those in the vehicle group (Figure 3.3, C), but this difference was not significant, as evidenced by the main effect of treatment [F(1,10)<1.0]. Body weight increased slightly, but significantly from PND 52 to 53 [F(1,10)=25.00, $p<0.001$]. The treatment by day interaction was not significant [F(1,10)=1.24, $p>0.05$].

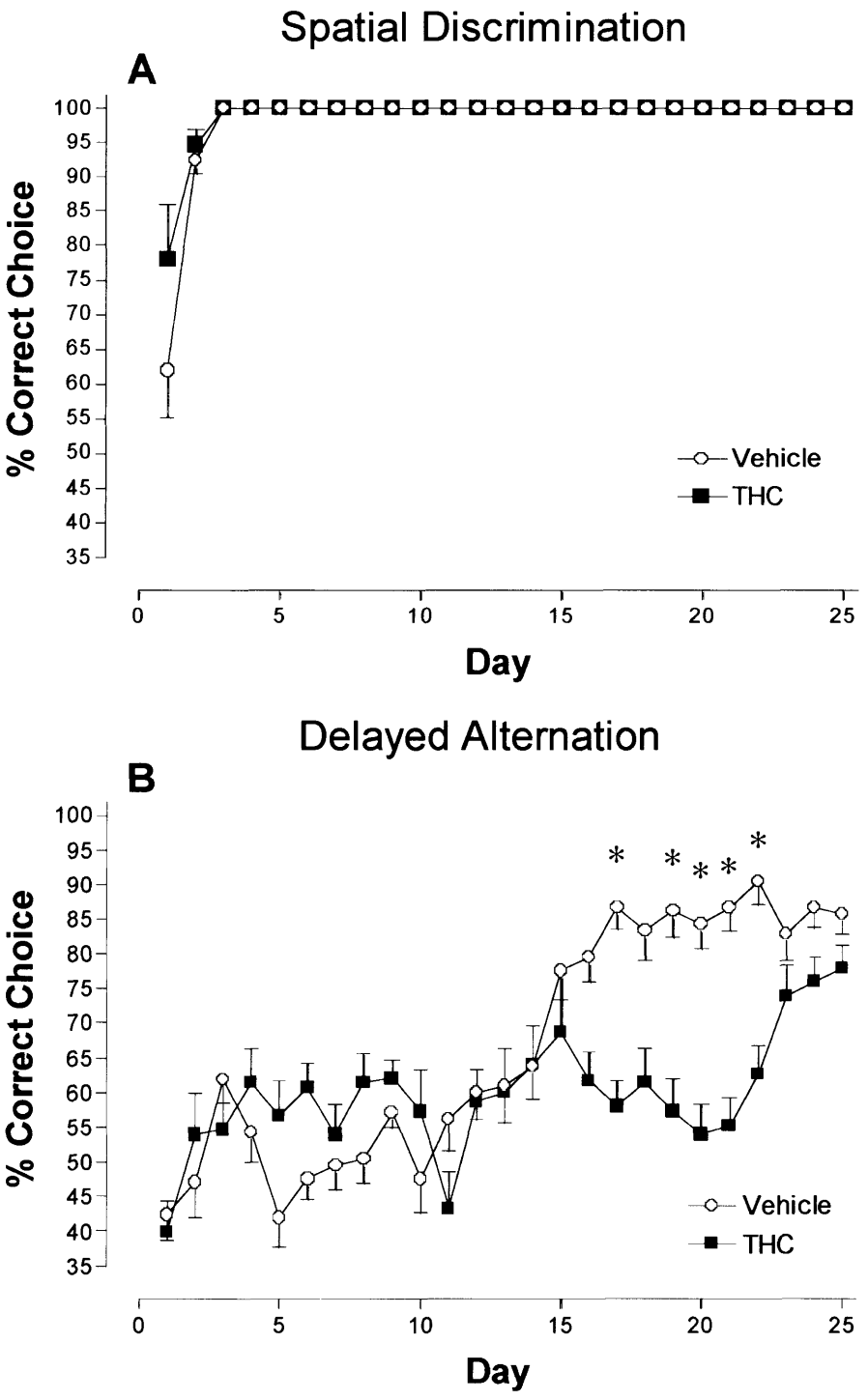

Figure 3.2 Mean percentage of correct choices in the spatial discrimination (A) and delayed alternation (B) components of the double Y-maze task during 25 test sessions of 30 trials each. Rats in the drug and vehicle group received either THC (5 mg/kg) or its vehicle from PND 4-14, followed by testing in the double Y-maze in adulthood (starting on PND 56). Error bars represent SEMs. *Vehicle group significantly different from THC group.

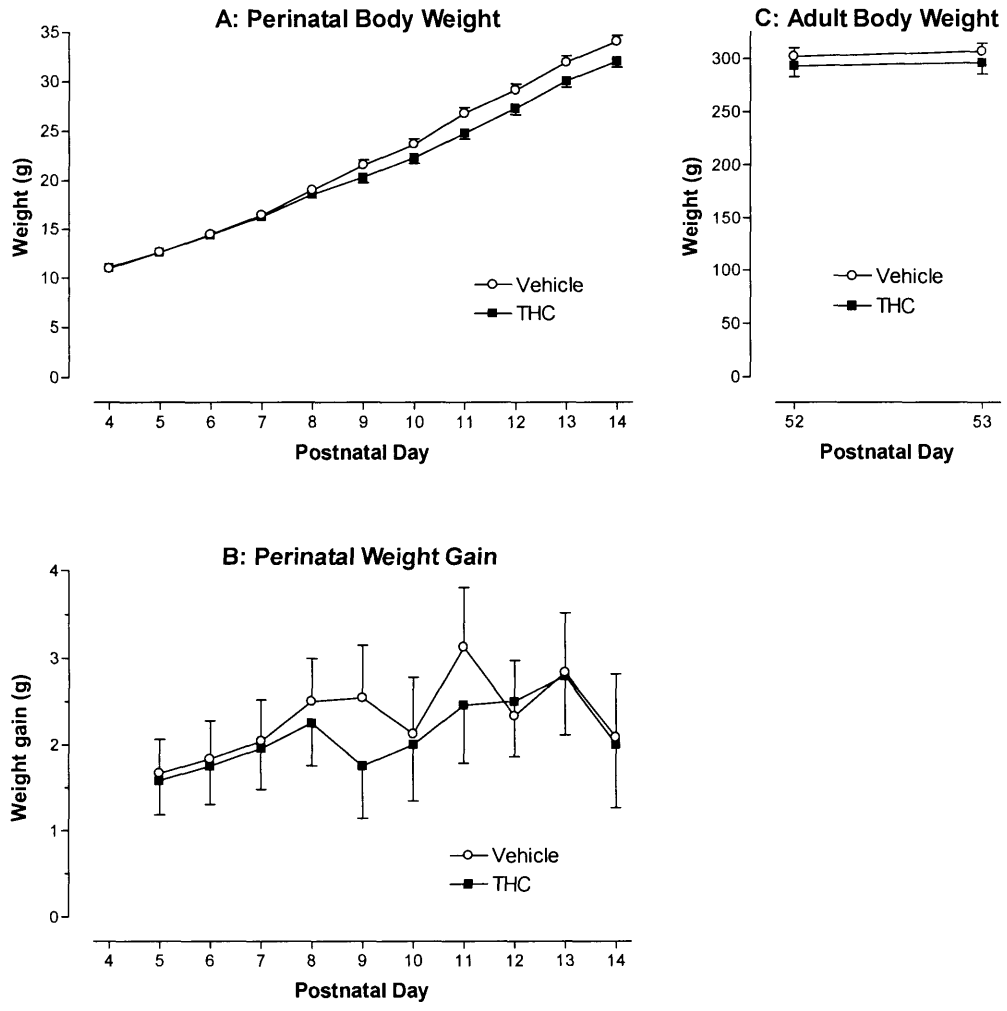

Figure 3.3 Body weight (A) and weight gain (B) during the 11-day drug administration period (PND 4-14) in rats receiving THC (5 mg/kg) or vehicle; and body weight (C) in adulthood (PND 52-53).

3.5 Discussion

Results revealed that daily exposure to THC from PND 4 to PND 14 led to a residual impairment in the delayed alternation component of a double Y-maze task when tested in early adulthood (i.e., PND 59-83). That is, rats exposed to THC during the early perinatal period committed more errors, and required longer to reach a high degree of proficiency--defined as 80% correct choices over 2 consecutive days--during adulthood in the delayed alternation task. No significant group differences were observed in the spatial discrimination component of the double Y-maze task, suggesting that the behavioural deficits observed cannot simply be attributed to drug-induced impairments in motivation, sensory-perceptual abilities, or motor function (Mallet & Beninger, 1993; Mason et al., 1999; Smith et al., 1994).

The present study adds to a growing literature showing that chronic cannabinoid exposure during the early developmental period leads to residual learning deficits in adulthood in humans (Fried et al., 2003; Leech et al., 1999; Richardson et al., 2002), or in rodents as assessed in maze learning tasks (Gianutsos & Abbatiello, 1972; Kawash et al., 1980) and in a passive avoidance test (Mereu et al., 2003). However, to the authors' knowledge, this study is the first to examine the residual cognitive effects of THC administration to rat pups. In previous studies (Abel, 1980) developing animals were exposed to a cannabinoid *in utero* by administering the drug to pregnant dams. However, confounds related to this *in utero* exposure approach possibly complicate the interpretation of results. For example, cannabinoid exposure may influence the postnatal behaviour of the mother, or

may induce maternal undernutrition which may in turn affect postnatal lactation (Abel, 1980).

Related to this, the present results revealed a subtle (yet significant) reduction in body weight by THC during the perinatal drug administration period (Figure 3.3, A). By PND 52-53 (i.e., the period immediately prior to behavioural testing), the difference in body weight between groups was no longer significant. A recent report (Fride et al., 2001) shows that the CB_1 cannabinoid receptor antagonist SR 141716 [N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide] dramatically reduces milk suckling in mouse pups, and this effect is attenuated by co-administration with the endogenous cannabinoid 2-AG. This same study also showed that growth in mouse pups was slightly increased by neonatal THC administration. It is not clear why THC produced opposite effects on body weight in these two studies, but the differential effects may simply be dose-related, or may be a function of differences in the pharmacological properties of THC and other cannabinoids. For example, SR 141716 has inverse agonist properties at CB_1 receptors (Landsman, Burkey, Consroe, Roeske, & Yamamura, 1997), and endogenous cannabinoids such as 2-AG also function as agonists at vanilloid receptors (for review see Howlett & Mukhopadhyay, 2000). Regardless, it remains possible that THC exposure in the present study may have affected the rat pups' feeding behaviour, which may have contributed to the long-term cognitive impairment in the delayed alternation task.

Our recent work revealed that chronic exposure to the synthetic cannabinoid receptor agonist CP 55,940 to adolescent, but not young adult rats, leads to impaired working memory and increased anxiety later in life

(O'Shea et al., 2004). These findings are in agreement with earlier studies showing that cannabinoid exposure in adolescent (i.e., 30-40 day old) rats (Fehr et al., 1976; Fehr et al., 1978; Stiglick & Kalant, 1982a; Stiglick & Kalant, 1982b; Stiglick & Kalant, 1983), but not adult rats (Stiglick & Kalant, 1985), resulted in impaired learning later in life. Taken together, these studies suggest that younger animals are more vulnerable to the cognitive impairing effects of cannabinoids. Results from the present study extend the vulnerable postnatal period to include the first few days after birth. This is significant because a significant amount of synaptic development occurs in the third trimester of pregnancy in humans, but in rats most synaptogenesis occurs in the first few days of postnatal life (Meyer & Kunkle, 1999). Although the neural mechanisms responsible for the residual cognitive impairing effects of THC observed here remain a matter for speculation, it is interesting to note that CB_1 cannabinoid receptors are present on axon terminal membranes during the early postnatal days (Morozov & Freund, 2003), and the endocannabinoid signalling system modulates gene expression of the neural adhesion molecule L1 (Gomez et al., 2003) .

Although choice accuracy for the delayed alternation component in the drug-treated group was not significantly below that of the vehicle-treated group until day 17 (see Figure 3.2, B), it is important to note that chance performance in a two-choice task is 50%. Our previous work (unpublished) using the double Y-maze task revealed that normal control animals typically remain near this chance level for many days, and then choice accuracy quickly rises. The finding that the groups did not differ significantly until well into the testing period therefore reflects that both groups were initially

performing near chance, as expected. The drug-induced cognitive deficit is arguably best shown by a significant delay in reaching a high level of performance.

A feature of the double Y-maze task is that different cognitive abilities are required to solve its two distinct components. The two tasks are similar in that they both involve a two-arm discrimination in a Y-maze for food reward. However, in trained animals only the delayed alternation component requires the use of working memory (Mallet & Beninger, 1993). The selective impairment in the delayed alternation component suggests that exposure to THC during the first few postnatal days produces a deficit specific to working memory. Unlike previous studies using animals to examine the influence of THC on cognitive performance, results from the present study were unlikely to be related to any gross impairments of motivation, motor function, or sensory-perceptual abilities. That is, impairments to any of these would likely have been expressed as a behavioural deficit in the spatial discrimination component.

The present study suggests that exposure to THC during the early postnatal period of life might compromise cognitive abilities that influence working memory in adulthood, which may manifest as subtle, but measurable learning difficulties. However, our findings alone are not sufficient to determine whether the residual learning deficits are permanent. Nonetheless, there is growing evidence that early life cannabis exposure, from the perinatal period through to adolescence, may lead to lasting and potentially irreversible cognitive deficits.

FOREWORD TO CHAPTERS 4-8

A manuscript based on Chapters 4-8 is under review in the *Journal of Psychopharmacology*. It was submitted on the 21st of July 2005, and we were notified by the journal on this same day that it has been sent off for peer review (ms no. is 114/05). Paul E. Mallet, my primary PhD supervisor, and Iain S. McGregor, my co-supervisor appear as co-authors on the paper which is titled "Repeated cannabinoid exposure during perinatal, adolescent, or early adult ages produces similar long-lasting deficits in object recognition and reduced social interaction". This research was supported by an ARC Discovery Grant and a UNERA.

CHAPTER 4: WORKING MEMORY IMPAIRMENTS IN ADULT RATS TREATED WITH CP 55,940 AT PERINATAL, ADOLESCENT, AND EARLY ADULT AGES

Abstract

Although many studies have examined the acute behavioural effects of cannabinoids in rodents, few have examined the lasting effects of cannabinoid exposure at different developmental ages. This study compared lasting effects of cannabinoid exposure with onset occurring at perinatal, adolescent, and adult ages. Twenty-four 4-day old (perinatal), twenty-four 30-day old (adolescent), and twenty-four 56-day old (young adult) male albino Wistar rats were injected with vehicle or incremental doses of the cannabinoid receptor agonist CP 55,940 once per day for 21 consecutive days (0.15, 0.20 or 0.30 mg/kg for 7 days per dose, respectively). Following a 28-day drug-free period, working memory was assessed using an object recognition task at delays of 2, 6 and 48 h. Results suggested that working memory was impaired in a delay-independent manner in rats treated with CP 55,940 at perinatal, adolescent, and adult ages. These results suggest that chronic exposure to a cannabinoid receptor agonist can lead to a lasting impairment of working memory regardless of age at exposure.

4.1 Introduction

Recent global estimates of cannabis use show that 163 million people use cannabis, making it the most widely produced, trafficked, and consumed illicit drug worldwide (United Nations Office on Drugs and Crime, 2003). Not surprisingly, cannabis is one of the most popularly used illicit drugs during pregnancy (Fried & Smith, 2001). Little, however, is known about the residual effects of cannabinoids that persist long after the drug has left the CNS (Pope et al., 1995), particularly the effects of perinatal exposure. Human and animal research shows that cannabinoids pass through the placental barrier during pregnancy (Blackard & Tennes, 1984; Hutchings et al., 1989), and can also be transferred through breast milk during lactation (Jakubovic et al., 1973). A major question that arises about *in utero* exposure is whether the effects reflect a direct action of cannabis on the fetus or represents consequences of the drug's effect on maternal organs (Wenger et al., 1992). This is arguably a major limitation of research on cannabinoid exposure occurring during pregnancy.

Of the few studies on perinatal cannabis exposure, one such study (Gianutsos & Abbatiello, 1972) treated pregnant Wistar rats with a cannabis extract (250 mg/kg.) on GD 8-11. Male and female offspring commenced Lashley III maze training on PND 65. Offspring of cannabis-treated rats required more trials to reach an acquisition criterion, committed more errors, and spent more time completing acquisition trials. Another study (Kawash et al., 1980) involved the administration of a cannabis extract (4.2 mg/kg) to pregnant Wistar rats on GD 2-6. On PND 22 randomly selected pups were tested in a Morris water maze. Cannabis-exposed rats required a significantly

greater number of trials to master maze learning. A more recent animal study (Mereu et al., 2003) involved WIN 55,212-2 exposure *in utero*. Drug-treated offspring consistently scored lower than controls on tests of memory retention throughout their lives. Further, WIN 55,212-2 disrupted glutamate release in the hippocampus and LTP, aspects of brain chemistry and electrical activity associated with memory and learning.

A longitudinal human study beginning in 1978 termed "The Ottawa Prenatal Prospective Study" (Fried et al., 2003) assessed the neurobehavioral and developmental effects of prenatal cannabis exposure from birth through adolescence. The assessment battery has included tests of general intelligence, achievement, and aspects of executive functioning such as cognitive flexibility in problem solving, focused attention, working memory, inhibition of prepotent responses, and monitoring, evaluating, and adjusting self-directed responses (Fried & Smith, 2001). To date, results generally suggest that *in utero* cannabis exposure is negatively associated with tasks requiring visual memory, analysis, and integration (Fried et al., 2003). Similarly, another human study (Richardson et al., 2002) assessed 10-year old children exposed to cannabis *in utero*. Assessment via a neuropsychological battery, focused on learning, memory, problem solving, mental flexibility, psychomotor speed, attention, and impulsivity, indicated that perinatal cannabis exposure was associated with learning and memory deficits, as well as impulsivity.

The effects of cannabis initiation occurring in and around the adolescent period also remain relatively unknown. This is despite the fact that human cannabis use is commonly initiated at this age (Scallet, 1991). It is

therefore of interest to determine whether adolescent cannabis use can produce lasting effects on cognitive function that continue to manifest in adult life. A few studies on rats of adolescent age have addressed 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 Wistar rats for 1-6 months, followed by a drug-free period of 1-2 months. Impairments on radial arm maze learning and motor coordination tasks were observed in rats treated with high doses for 6 months. Another study (Stiglick & Kalant, 1982b) involved a drug-free period of 2-3 months and found that rats treated with THC (20 mg/kg) for 3 or 6 months showed deficits in DRL and locomotor activity.

More recently, a study (Schneider & Koch, 2003) involved chronic exposure to WIN 55,212-2 (1.2 mg/kg) in either adolescent (40-day old) or adult (70-day old) male rats over a 25-day period. Following a 10 day drug-free period, rats were assessed on behavioural tasks including the object recognition task. Deficits in working memory were apparent in rats exposed to WIN 55,212-2 in the adolescent but not adult period of life.

A human study (Ehrenreich et al., 1999) assessed visual scanning along with other attentional functions in adult cannabis users (mostly male) who had been early (before age 16) or late onset users (after age 16). The results showed that early onset cannabis users had attention deficits specific to visual scanning. Another human study (Schwartz et al., 1989) found that cannabis-using adolescents (mostly male) maintained working memory deficits when assessed up to 6 weeks after the last drug administration.

Although much cannabis research is conducted with adult populations, primarily investigating the acute effects of cannabis, there is a dearth of information on the residual consequences of cannabis exposure when onset occurs in the early adult period of life. Whilst some studies (Ehrenreich et al., 1999; Pope, 2002) have examined early versus late cannabis exposure occurring in adolescence, these studies have found no evidence of cognitive deficits approaching an early adult age.

A few animal studies have also addressed cannabinoid exposure at adult onset. One study (Stiglick & Kalant, 1985) exposed 70-day old adult male Wistar rats to THC for 3 months. Following a 1- to 4-month drug-free period, no deficits in performance were found when assessed 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. A study by Deadwyler et al. (1995) exposed adult male Sprague-Dawley rats (approximately 70-days old) to THC (10 mg/kg) followed by evaluation on a spatial discrimination version of a DMTS short-term memory task. Exposure to THC initially led to a severe disruption of DMTS performance, which disappeared following 30 to 35 days of THC-exposure. Withdrawal from the drug after this period produced a slight reduction in performance at all delays, which dissipated within 2 days. No subsequent residual effects of the 35-day THC-exposure were apparent up to 15 days after the last drug administration. Similarly, a study by Nakamura et al. (1991) on adult (56-day old) male Wistar rats assessed both the acute and chronic effects of THC administration on memory (eight-arm radial maze). Both acute (1 x 1.25 mg/kg dose) and chronic (5 mg/kg; for a 90-day period) administration affected working

memory, but not reference memory in this task. The memory deficits were reversed following a 30-day drug-free period.

The current study builds on a previous study in the author's laboratory (O'Shea et al., 2004), which compared residual cannabinoid effects when exposure occurred in either adolescence or early adulthood. Adolescent (30-day old) and adult (56-day old) female Wistar rats were injected with vehicle or incremental doses of CP 55,940 daily for 21 consecutive days (0.15, 0.20 or 0.30 mg/kg), followed by a 21-day drug-free period. Working memory was assessed using the object recognition task, and anxiety was assessed using a social interaction test. Poorer working memory and decreased social interaction (increased social anxiety) was observed in adolescent but not adult CP 55,940-treated rats.

Although some studies have investigated the residual effects of cannabinoids, systematic comparisons of age at onset of exposure have not been conducted to date. The purpose of the current study was to assess the possible lasting effects of chronic cannabinoid exposure on working memory in rats exposed to the synthetic cannabinoid CP 55,940 at major developmental ages. Onset of cannabinoid exposure occurred at PND 4, 30, or 56 corresponding with perinatal, adolescent, and early adult ages, respectively. 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 et al., 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). Likewise, there is mounting evidence that the onset of cannabis exposure at an adolescent age is associated with lasting cognitive change (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 was associated with lasting behavioural change (O'Shea et al., 2004). This former study however, involved female rats, and there is mounting evidence that the most pronounced behavioural and neurological change due to cannabinoid exposure is demonstrated in males (for review see Fernández-Ruiz et al., 1992; Moreno et al., 2003; Navarro et al., 1996).

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). More specific to this current study, object recognition has been used to assess the effects of

cannabinoid receptor agonists such as CP 55,940 (Kosiorek, Hryniewicz, Bialuk, Zawadzka, & Winnicka, 2003) and WIN 55,212-2 (Schneider & Koch, 2002, 2003).

There are a variety of human and non-human models of memory, which encompass memory domains such as working, reference, declarative, procedural, episodic, and semantic memory, to name a few (Oscar-Berman, 1991). In terms of the object recognition task itself, there is wide consensus that this particular animal model measures working memory (see Dodart, Mathis, & Ungerer, 1997; Ennaceur & Delacour, 1988; Ennaceur et al., 1989; Ennaceur & Meliani, 1992; Ennaceur, Neave, & Aggleton, 1997), as it refers to trial-dependent memory as opposed to reference memory (trial-independent). It is devoid of a reference memory component as it does not involve rule learning. Further, this task does not require the use of positive or negative reinforcers, such as food or electric shock, which are characteristic of many other animal models of "memory" (Ennaceur & Delacour, 1988). Instead, this task takes advantage of the rats' innate tendency to explore novel rather than familiar objects, where a reduced ability to discriminate between objects is indicative of working memory dysfunction. "Working memory" in this task is therefore defined as the immediate retention of information needed to respond to a current task or activity (Honig, 1978).

The object recognition task traditionally 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 the delays increase (Ennaceur & Delacour, 1988). The measurement of locomotor activity was introduced as an adjunct to this task to determine whether drug exposure results in long-

term alterations in physical performance. In T1, a rat is presented with two identical objects. In T2, one of the objects is replaced with a novel one. 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.

In T1 of this experiment no differences in exploration of the identical objects was expected for all groups. In T2, it was expected that the immature cannabinoid groups (i.e., the perinatal and adolescent CP 55,940-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 cannabinoid group could manifest a decrease in the ability to discriminate between objects relative to controls. In terms of locomotor activity, no difference between all groups was anticipated.

4.2 Materials and Method

4.2.1 Subjects

Seventy-two male Wistar rats were used. Twenty-four rats were 4 days old (perinatal), 24 were 30 days old (adolescent), and 24 were 56 days old (adult). At each developmental age, 12 of these rats comprised the drug-exposed group and the other 12 served as vehicle controls. At the start of treatment perinatal rats weighed 9-12 g, adolescent rats weighed 77-123 g, and adult rats weighed 269-348 g. Animals had *ad libitum* access to food and water and were group-housed in a temperature- and humidity-controlled colony room maintained on a 12-h light/12-h dark cycle.

The postpartum protocol used in the previous chapter (Double Y-Maze) was used for perinatal groups. That is, At PND 2, 40 albino Wistar rat pups

were pooled, sexed, and randomised into four groups of eight males and two females each. Each group then was allocated randomly to one of the postpartum dams. On PND 21 pups were weaned and female pups were culled. Of these 40 rats, 24 male rats were used.

4.2.2 Drug Preparation and Administration

CP 55,940 [(-)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl) cyclohexanol; Tocris Cookson, UK] was dissolved in a vehicle containing 15 µl Tween 80 (polyoxyethylene sorbitan monooleate, ICN Biochemicals) per 2 ml physiological saline. All injections were administered s.c. (5 ml/kg) for perinatal rats or intraperitoneal (i.p.) (1 ml/kg) for adolescents and adults. Rats in the drug-treated groups received increasing doses of CP 55,940 for 21 consecutive days (0.15, 0.20 and 0.30 mg/kg for 7 days at each dose), while the control groups received similar exposure to the drug's vehicle. Gradually increasing doses were employed to partially counter the development of drug tolerance.

4.2.3 Apparatus and Procedure

Following a 28-day drug-free period rats were tested in the object recognition task. At the onset of testing, perinatal groups were now 53 days old, adolescent groups were 79 days old, and adult groups were 105 days old. The experimental chamber was a clear Perspex box (610 x 260 x 400 mm). Experiments were conducted under low light conditions. Each trial was videotaped using a black and white charged-coupled device (CCD) camera with infrared illumination. Locomotor activity was measured via a passive infrared sensor connected to a computer with custom software to detect and record time spent in motion.

Objects used included coffee mugs, tin cans, plastic bottles, rice bowls, red plastic boxes, and tubs of hair gel. To eliminate any possible influence of olfactory cues, three copies of each object were used: two were used in the first trial, and the remaining object was used in the second trial. Objects were washed with Pyroneg detergent (Diversey Lever, Australia) prior to each trial, and the experimental chamber floor and walls were wiped between trials with a 1:10 vinegar:water solution. The assignment of objects used in any given trial was counterbalanced such that object combinations were distributed equally across groups.

On the day prior to testing rats were habituated to the experimental chamber for two non-consecutive 2-min periods to reduce experimental chamber novelty. Formal testing began the next day. In the first trial (T1) each rat was presented with two identical objects for 10 min. The purpose of this trial was simply to provide an opportunity for the rats to explore two identical copies of an object. During the second trial (T2), which occurred 2, 6 or 48 h later, the rats were again presented with two objects for 10 min. This time one object was novel, and the other was a triplicate of the original object presented in T1. All rats were tested at all three delays. Delays of 2, 6, or 48 h were counterbalanced and distributed equally across both vehicle- and CP 55,940-treated groups. Object exploration was said to occur when the rat's snout was directed within 2 cm of the object. Climbing on or sitting on the object was not recorded. An observer blind to the group allocations manually scored the video recordings of each trial using the software package ODLog (Macropod Software, 2001; www.macropodsoftware.com).

4.3. Statistical Analysis

4.3.1 Object recognition

The time spent investigating objects during T1 was calculated by summing the time spent exploring each identical object to produce a single score. These values were then compared using three (one for each age group) two-way ANOVAs (treatment x delay) with repeated measures on the delay factor. Where a main effect of delay was observed, Bonferroni-adjusted *t*-tests were used to compare each delay to the others. A three-way (age x treatment x delay) ANOVA with repeated measures on the delay factor was also used to examine the influence of age across groups. Groups were then compared using *post hoc* Tukey tests where significant main effects of age were found. Bonferroni-adjusted *t*-tests were used to compare delay main effects.

The percentage of time spent investigating the novel object in T2 was calculated according to the formula N ÷ (N + F) x 100 where N and F represented time spent investigating the novel and familiar objects, respectively. These values were then analysed in the same way described for the T1 data.

4.3.2 Locomotor activity

Time spent in motion was recorded during all sessions. These values were then compared across experimental conditions using treatment by delay ANOVAs, with repeated measures on the delay factor, for each age group at T1 and T2. Bonferroni-adjusted *t*-tests were conducted where main effects of delay were observed. Age by treatment by delay ANOVAs, with repeated measures on the delay factor, were used to examine the effects of age at T1

and T2. Where main effects of age were observed, *post hoc* Tukey tests were conducted. Bonferroni-adjusted *t*-tests were used where a main effect of delay was observed. Where an age by delay interaction was observed, one-way ANOVAs compared age at each level of delay, followed by *post hoc* Tukey tests where ANOVA findings were significant.

In all analyses, where the ANOVA assumptions were not met, randomisation tests of scores were conducted using NPFact version 1.0. In all cases the randomisation tests supported the ANOVA findings so for ease of interpretation only the ANOVA results are presented. All *t*-tests, ANOVAs, and *post hoc* tests were conducted using SPSS 12.0.1 for Windows.

4.4 Results

4.4.1 Object Recognition.

4.4.1.1 Trial 1

Perinatal CP 55,940- and vehicle-treated groups spent roughly equivalent amounts of time investigating the identical objects. Delays did not appear to influence object investigation. The means for treatment groups averaged across delays are presented in Figure 4.1. In addition, the means for the treatment groups are presented separately for 2, 6, and 48 h delays in Figure 4.2. The two-way ANOVA (treatment x delay) revealed that the main effect of treatment [$F(1,22)<1.0$], the delay main effect [$F(2,44)=1.40$, $p>0.05$], and the treatment x delay interaction [$F(2,44)<1.0$] were not significant (for statistics outputs see Appendix A5 & A6 and for data see Appendix B3).

Adolescent CP 55,940 and vehicle groups spent similar amounts of time investigating identical objects. The means for treatment groups averaged across delays are presented in Figure 4.3. The means for the treatment

groups are presented separately for each delay in Figure 4.4. The main effect of treatment [$F(1,22)<1.0$], and the treatment x delay interaction [$F(2,44)=1.13$, $p>0.05$] were not significant. However, the delay main effect was significant [$F(2,44)=5.22$, $p<0.01$] (for statistics outputs see Appendix A7 & A8 and for data see Appendix B4). Despite obtaining a significant main effect of delay, Bonferroni-adjusted *t*-tests comparing each delay to all others revealed no significant differences.

Adult CP 55,940 and vehicle groups also showed similar object investigation during T1. The means for treatment groups averaged across delays are presented in Figure 4.5. The means for the treatment groups are presented separately for each delay in Figure 4.6. The main effect of treatment [$F(1,22)<1.0$], main effect of delay [$F(2,44)<1.0$], and the treatment x delay interaction [$F(2,44)<1.0$] were not significant (for statistics outputs see Appendix A9 & A10 and for data see Appendix B5).

The three-way ANOVA (age x treatment x delay) revealed significant main effects of age [$F(2,66)=11.03$, $p<0.001$], and delay [$F(2,132)=3.84$, $p<0.05$]. Neither the treatment main effect, nor any of the other interactions, was significant. Tukey *post hoc* tests comparing each age to the other two revealed that adult rats spent significantly less time investigating objects during T1 relative to both perinatal and adolescent rats (see Figure 4.1, 4.3, and 4.5). Despite obtaining a significant main effect of delay, Bonferroni-adjusted *t*-tests comparing delays revealed no significant differences during T1.

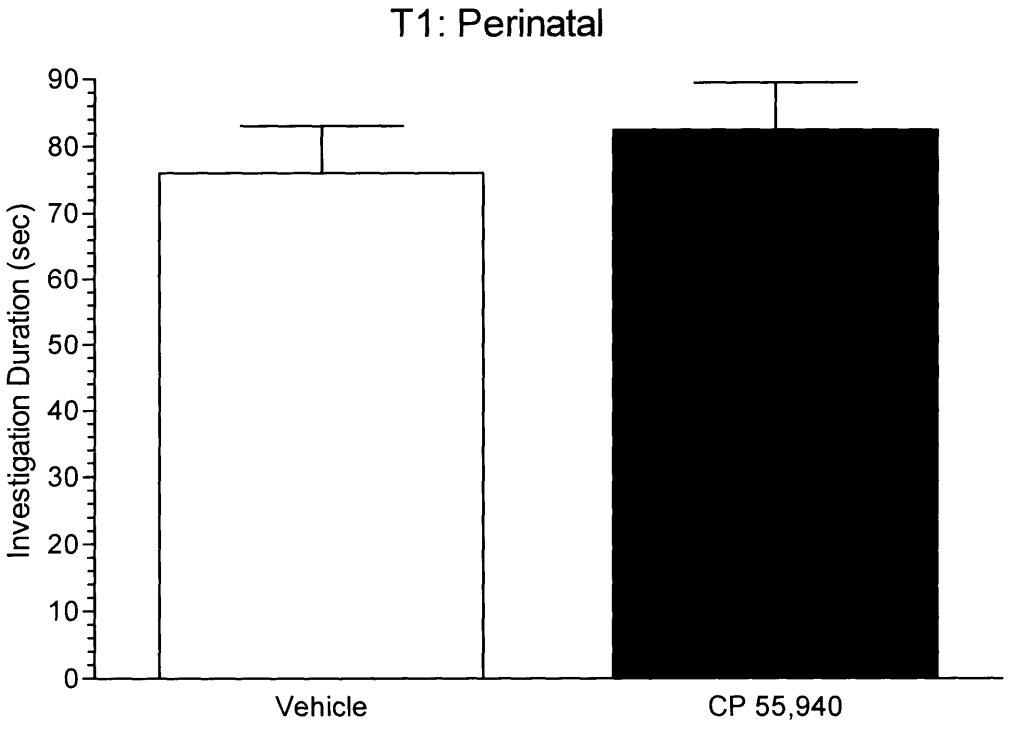

Figure 4.1 Time (sec) spent exploring identical objects in T1 for perinatal rats in vehicle- and CP 55,940-treatment groups. Data are averaged across delays.

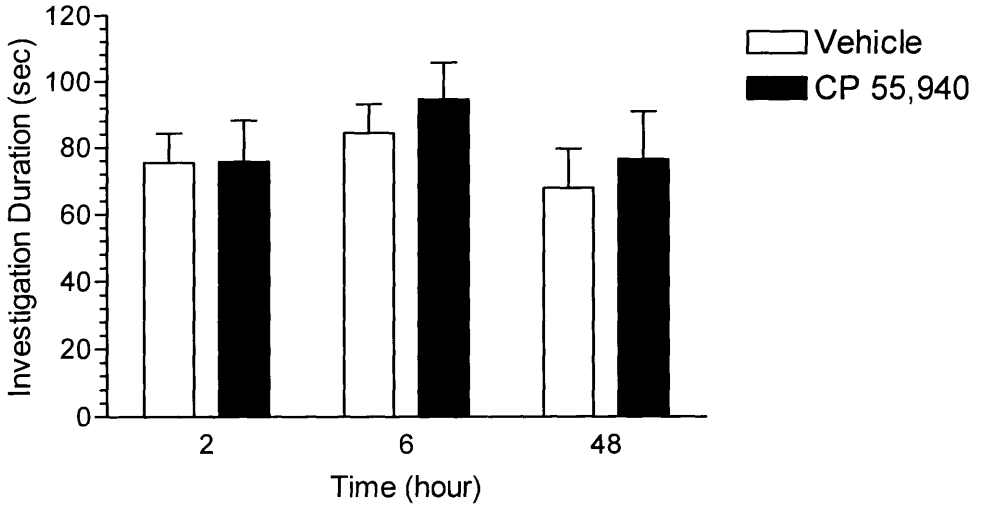

Figure 4.2 Time (sec) spent exploring identical objects in T1 for perinatal rats in vehicle- and CP 55,940-treatment groups. Data are presented separately for the 2-, 6-, and 48-h delays.

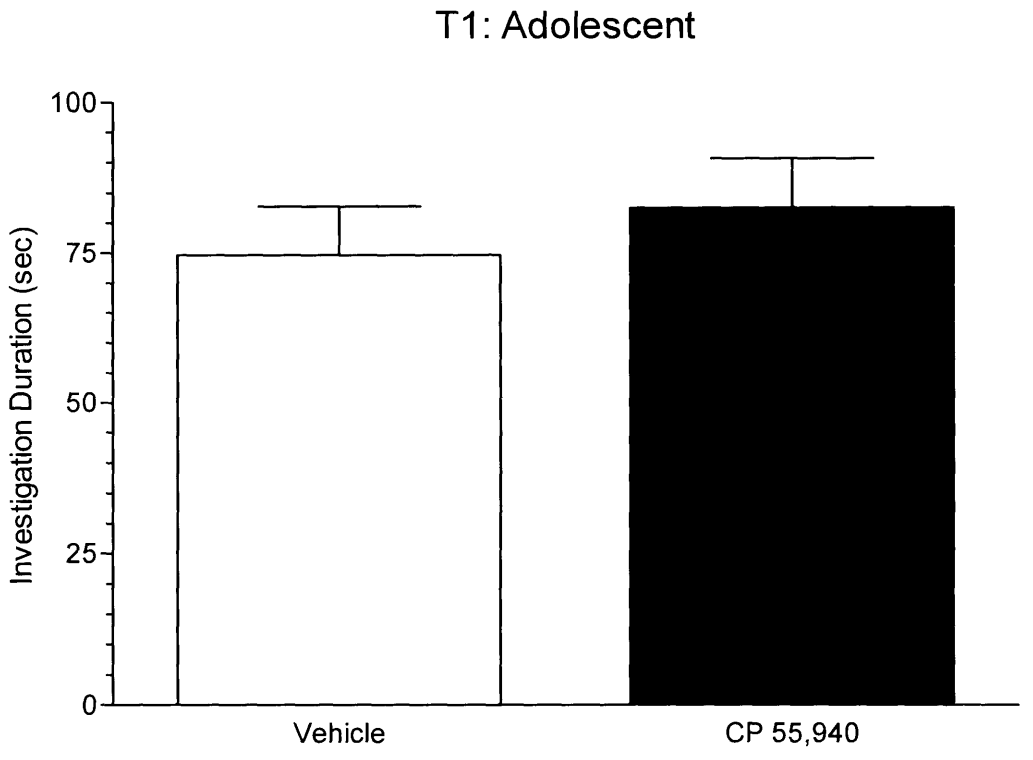

Figure 4.3 Time (sec) spent exploring identical objects in T1 for adolescent rats in vehicle- and CP 55,940-treatment groups. Data are averaged across delays.

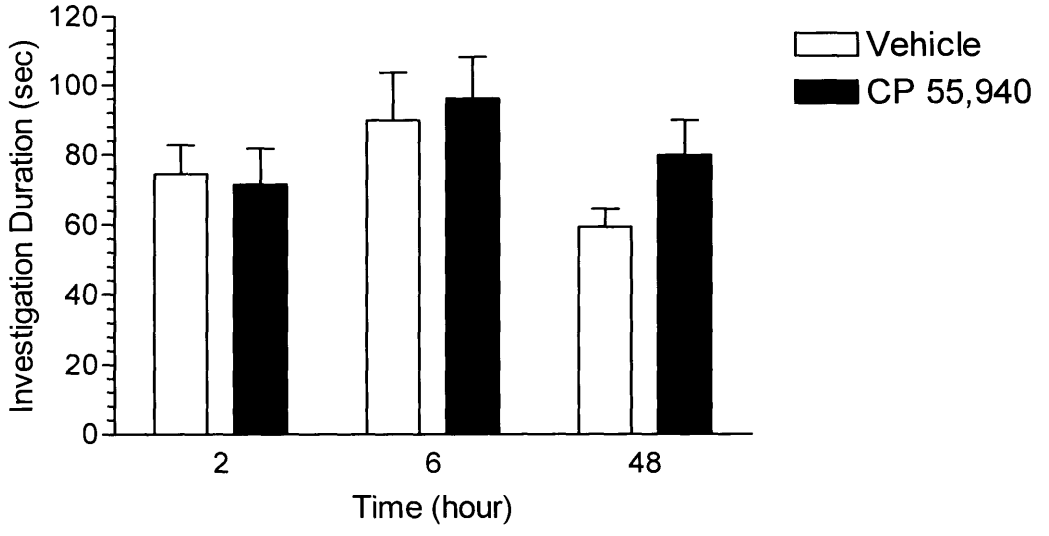

Figure 4.4 Time (sec) spent exploring identical objects in T1 for adolescent rats in vehicle- and CP 55,940-treatment groups. Data are presented separately for the 2-, 6-, and 48-h delays.

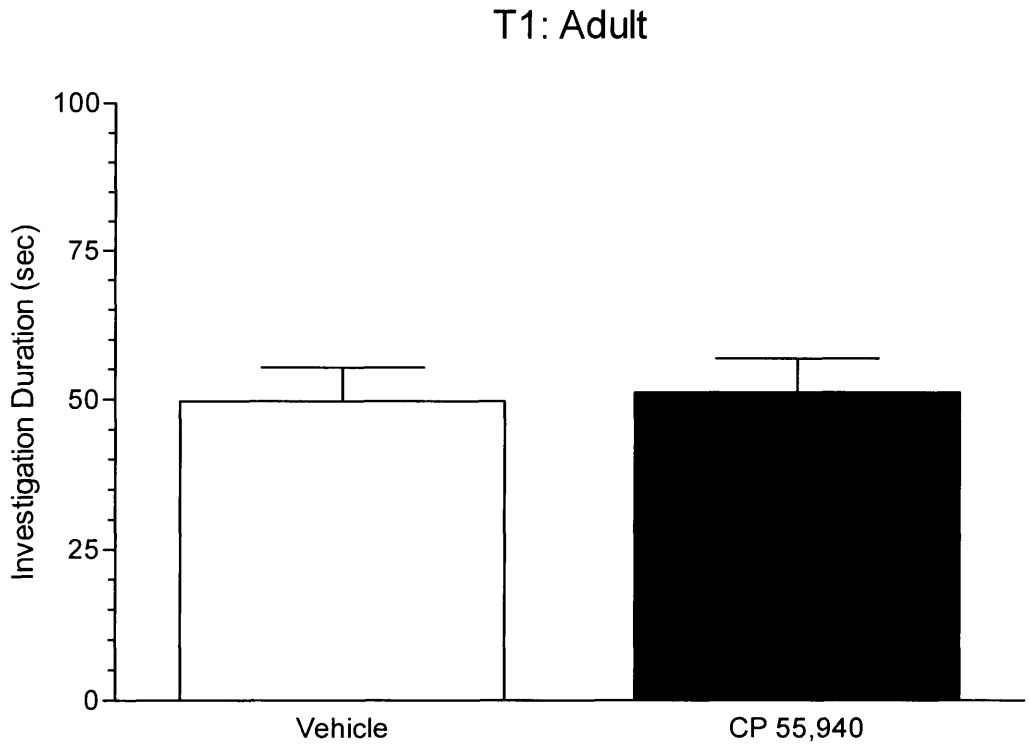

Figure 4.5 Time (sec) spent exploring identical objects in T1 for adult rats in vehicle- and CP 55,940-treatment groups. Data are averaged across delays.

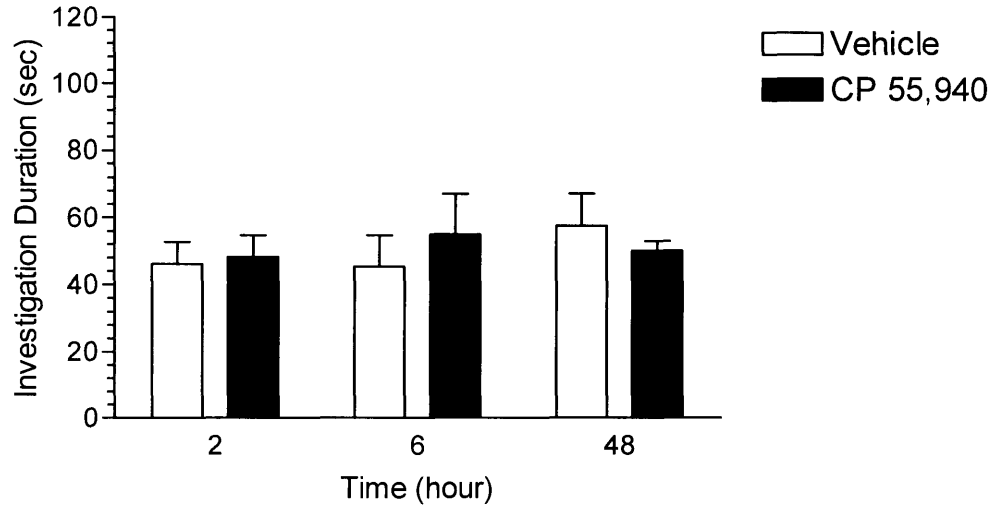

Figure 4.6 Time (sec) spent exploring identical objects in T1 for adult rats in vehicle- and CP 55,940-treatment groups. Data are presented separately for the 2-, 6-, and 48-h delays.

4.4.1.2 Trial 2

Within perinatal treatment groups, the preference for the novel objects was lower in the CP 55,940-treated group compared to controls. See Figure 4.7 for averaged means between groups and Figure 4.8 for means presented at 2, 6, and 48 h delays. The two-way (treatment x delay) ANOVA showed that the main effect of treatment was significant [$F(1,22)=4.51$, $p<0.05$]. However, the delay main effect [$F(2,44)=1.59$, $p>0.05$], and the treatment x delay interaction [$F(2,44)<1.0$] were not significant (see Appendix A11 & A12 for outputs and Appendix B6 for data).

Within adolescent groups, the preference for novel objects was also lower in the CP 55,940-treated group compared to controls. See Figure 4.9 for averaged means between groups, and Figure 4.10 for means presented at delays. The main effect of treatment was significant [$F(1,22)=9.29$, $p<0.01$]. The delay main effect [$F(2,44)<1.0$], and the treatment x delay interaction [$F(2,44)<1.0$] were not significant (see Appendix A13 & A14 for outputs and Appendix B7 for data).

Within adult groups, the CP 55,940-treated group showed a lower preference for the novel objects compared to controls. See Figure 4.11 for average means between groups, and Figure 4.12 for means across delays. The main effect of treatment was significant [$F(1,22)=4.66$, $p<0.05$]; however, the delay main effect [$F(2,44)=1.27$, $p>0.05$], and the treatment x delay interaction [$F(2,44)<1.0$] were not significant (see Appendix A15 & A16 for outputs and Appendix B8 for data). The three-way ANOVA (age x treatment x delay) revealed a main effect of treatment [$F(1,66)=18.13$, $p<0.001$], but none of the other main effects or interactions were significant.

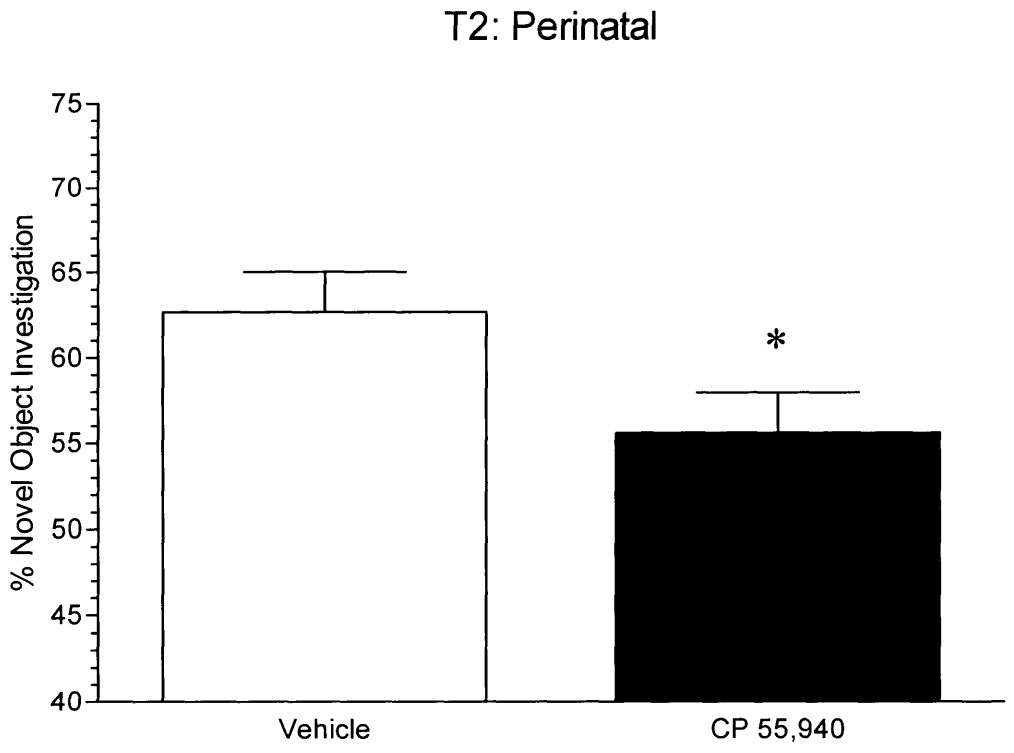

Figure 4.7 Percentage of time investigating the novel object during T2 for perinatal rats in vehicle- and CP 55,940-treatment groups. Data are averaged across delays. *CP 55,940 rats showed a significantly lower preference for novel objects compared to controls.

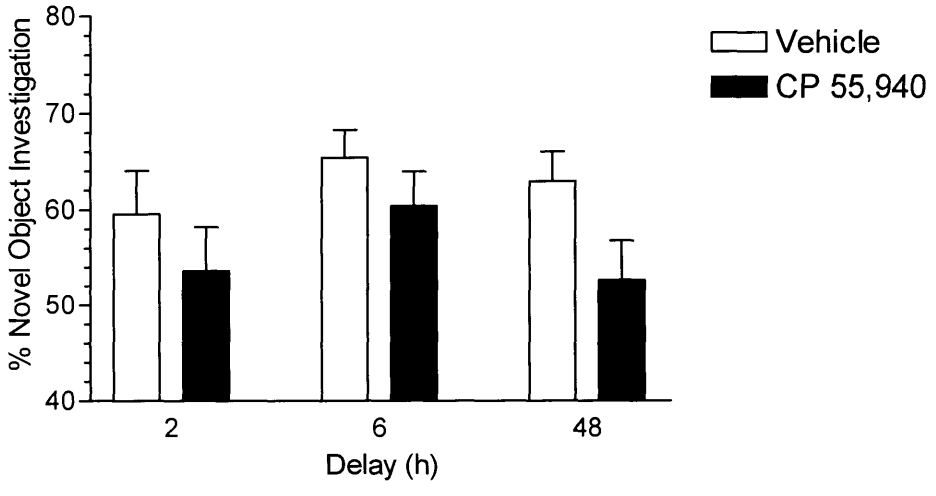

Figure 4.8 Percentage of time investigating the novel object in T2 for perinatal rats in vehicle- and CP 55,940-treatment groups. Data are presented separately for the 2-, 6-, and 48-h delays.

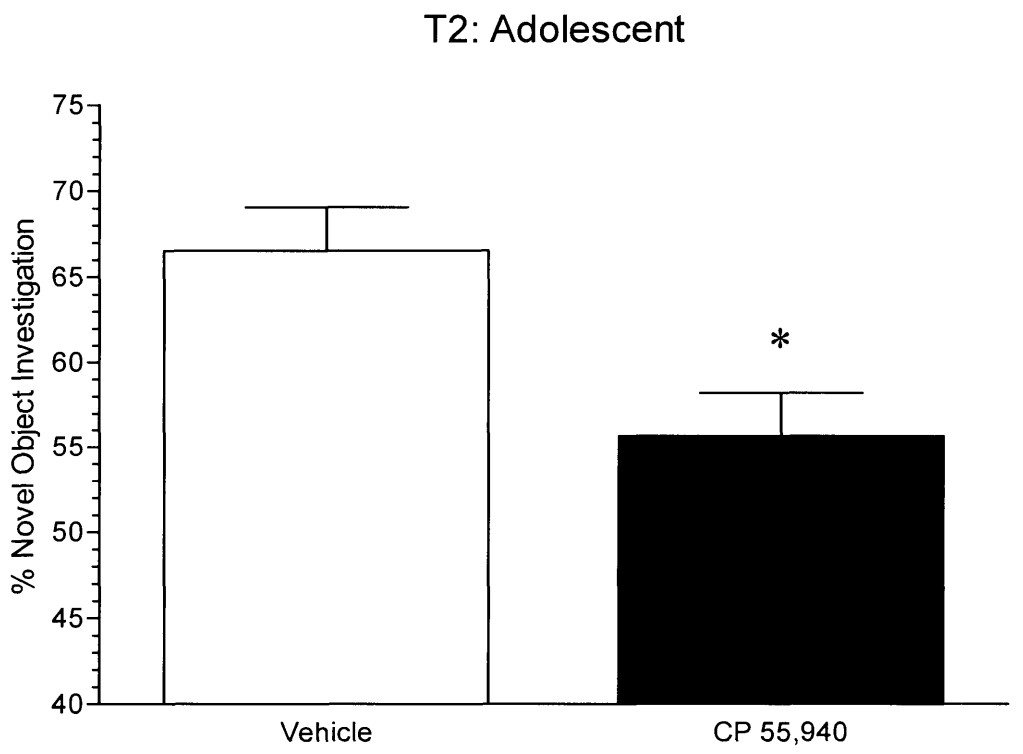

Figure 4.9 Percentage of time investigating the novel object during T2 for adolescent rats in vehicle- and CP 55,940-treatment groups. Data are averaged across delays. *CP 55,940 rats showed a significantly lower preference for novel objects compared to controls.

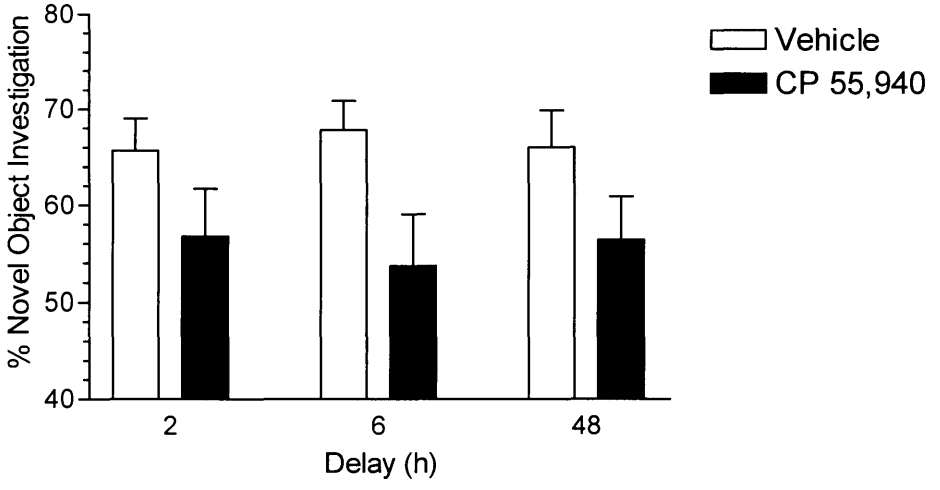

Figure 4.10 Percentage of time investigating the novel object in T2 for adolescent rats in vehicle- and CP 55,940-treatment groups. Data are presented separately for the 2-, 6-, and 48-h delays.

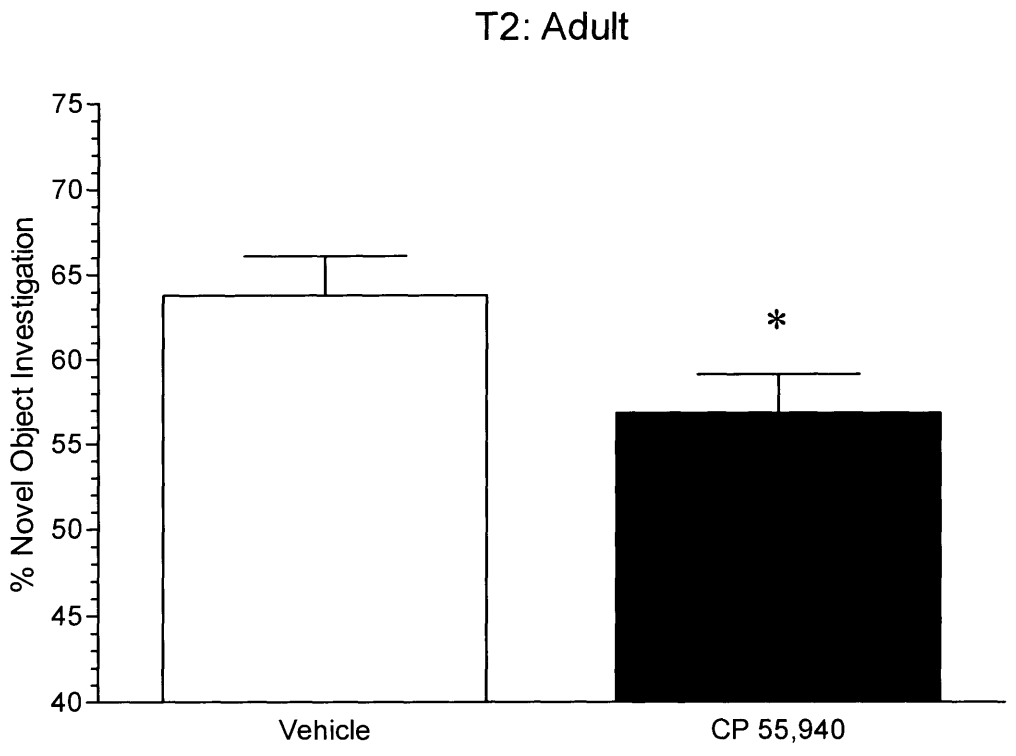

Figure 4.11 Percentage of time investigating the novel object during T2 for adult rats in vehicle- and CP 55,940-treatment groups. Data are averaged across delays. *CP 55,940 rats showed a significantly lower preference for novel objects compared to controls.

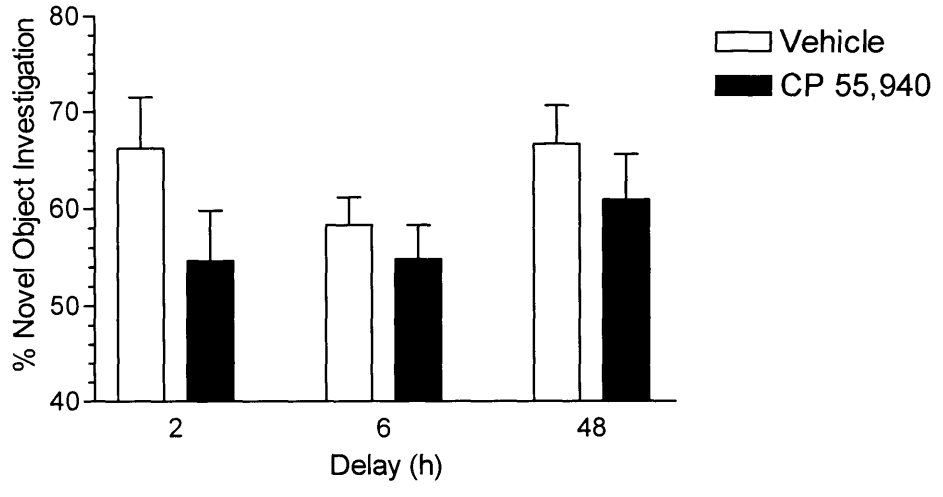

Figure 4.12 Percentage of time investigating the novel object in T2 for adult rats in vehicle- and CP 55,940-treatment groups. Data are presented separately for the 2-, 6-, and 48-h delays.

4.4.2 Locomotor Activity.

4.4.2.1 Trial 1

Locomotor activity did not differ significantly across delays or treatments during T1. However, in general the rats treated in adulthood, and therefore tested at a later age, were less active than the other two age groups. See Table 4.1.

In perinatal treatment groups, the two-way (treatment x delay) ANOVA revealed that the main effect of treatment [$F(1,22)=2.55$, $p>0.05$], main effect of delay [$F(2,44)=1.02$, $p>0.05$], and the treatment x delay interaction [$F(2,44)<1.0$] were not significant (for statistics outputs see Appendix A17 & A18 and for data see Appendix B9).

In adolescent treatment groups, the two-way ANOVA (treatment x delay) showed that the main effect of treatment [$F(1,22)=2.06$, $p>0.05$], main effect of delay [$F(2,44)=1.51$, $p>0.05$], and the treatment x delay interaction [$F(2,44)<1.0$] were not significant (for statistics outputs see Appendix A19 & A20 and for data see Appendix B10).

In adult treatment groups, the two-way ANOVA (treatment x delay) showed that the main effect of treatment [$F(1,22)<1.0$], main effect of delay [$F(2,44)=1.41$, $p>0.05$], and the treatment x delay interaction [$F(2,44)<1.0$] were not significant (for statistics outputs see Appendix A21 & A22 and for data see Appendix B11).

The three-way ANOVA (age x treatment x delay) revealed that the main effect of age [$F(2,66)=15.62$, $p<0.001$] was significant. *Post-hoc* Tukeys tests comparing ages revealed that locomotor activity in adult rats was

significantly lower than that of both perinatal and adolescent rats. None of the other main effects or interactions was significant.

Table 4.1 Mean±SEM time (sec) spent in locomotor activity in Trial 1 (T1) for perinatal, adolescent, and adult rats 2-, 6- and 48-h prior to the recognition test.

Treatment Age	Treatment	2 h	6 h	48 h
Perinatal	Vehicle	317.7 ± 11.2	313.8 ± 12.4	328.8 ± 8.2
	CP 55,940	334.3 ± 9.3	320.7 ± 11.2	338.3 ± 10.5
Adolescent	Vehicle	321.3 ± 14.1	316.6 ± 14.9	340.6 ± 11.3
	CP 55,940	323.9 ± 13.2	343.2 ± 12.0	350.3 ± 10.0
Adult	Vehicle	286.0 ± 20.5	304.5 ± 12.9	292.2 ± 7.3
	CP 55,940	281.7 ± 8.3	293.0 ± 12.7	302.4 ± 11.9

4.4.2.2 Trial 2

During T2, locomotor activity was similar in rats treated during the perinatal and adolescent periods, but rats treated during adulthood were slightly less active than the younger age groups. See Table 4.2.

In perinatal treatment groups, the two-way (treatment x delay) ANOVA revealed that the main effect of treatment [$F(1,22)<1.0$], main effect of delay [$F(2,44)=1.71$, $p>0.05$], and the treatment x delay interaction [$F(2,44)<1.0$] were not significant (for statistics outputs see Appendix A23 & A24 and for data see Appendix B12).

In adolescent treatment groups, the two-way ANOVA (treatment x delay) showed that the main effect of treatment [$F(1,22)=2.92$, $p>0.05$], main effect of delay [$F(2,44)=2.31$, $p>0.05$], and the treatment x delay interaction [$F(2,44)<1.0$] were not significant (for statistics outputs see Appendix A25 & A26 and for data see Appendix B13).

In adult treatment groups, the main effect of treatment [$F(1,22)<1.0$], and the treatment x delay interaction [$F(2,44)=1.36$, $p>0.05$] was not significant; however, the main effect of delay [$F(2,44)=10.41$, $p<0.001$] was significant (for statistics outputs see Appendix A27 & A28 and for data see Appendix B14). Bonferroni-adjusted *t*-tests comparing each delay to the other two revealed that locomotor activity at the 2-h delay was significantly lower than that at the 48-h delay [$t(46)=2.94$, $p<0.05$]. None of the other comparisons was significant. The interaction and treatment main effect were not significant.

The three-way ANOVA (age x treatment x delay) revealed that the age main effect [$F(2,66)=17.06$, $p<0.001$], delay main effect [$F(2,132)=8.42$, $p<0.001$], and age by delay interaction [$F(4,132)=2.71$, $p<0.05$] were significant. The treatment main effect and the other interactions were not significant. *Post-hoc* Tukeys tests on age revealed that the locomotor activity of adult rats was significantly lower than that of both perinatal and adolescent rats. Bonferroni-adjusted *t*-tests comparing delays showed that locomotor activity at the 2-h delay ($M=293.5$) was significantly lower than activity at 6-h delay [$M=314.9$; $t(142)=2.62$, $p<0.05$] and at the 48-h delay [$M=312.9$; $t(142)=2.53$ $p<0.05$]. One-way ANOVAs comparing ages at each level of delay showed that there were significant differences between age groups at the 2-h [$F(2,71)=14.01$, $p<0.001$], 6-h [$F(2,71)=7.79$, $p<0.01$], and 48-h [$F(2,71)=6.98$, $p<0.01$] delays. *Post-hoc* Tukeys tests revealed that the locomotor activity of adult rats was significantly lower than that of perinatal and adolescent rats at both the 2-and 6-h delay. At the 48-h delay, the

locomotor activity of adolescent rats was significantly higher than that of the adult rats.

Table 4.2 Mean±SEM time (sec) spent in locomotor activity in Trial 2 (T2) for perinatal, adolescent, and adult rats following 2-, 6- and 48-h delays.

Treatment Age	Treatment	2 h	6 h	48 h
Perinatal	Vehicle	319.1 ± 9.8	327.9 ± 9.3	311.9 ± 12.8
	CP 55,940	316.5 ± 9.7	337.3 ± 9.5	317.4 ± 10.1
Adolescent	Vehicle	301.3 ± 12.4	316.9 ± 14.3	318.9 ± 9.2
	CP 55,940	317.3 ± 10.3	330.2 ± 9.0	341.6 ± 8.7
Adult	Vehicle	259.5 ± 22.3	299.9 ± 10.1	289.3 ± 8.2
	CP 55,940	247.2 ± 11.5	277.5 ± 16.3	298.2 ± 9.1

4.5 Discussion

It was initially hypothesised that the immature cannabinoid groups (i.e., perinatal and adolescent) would show working memory deficits in the object recognition task relative to immature controls. In adult groups, it was also anticipated that the cannabinoid group could manifest working memory deficits. The results showed that CP 55,940-treated rats at all ages exhibited a decreased ability to discriminate between novel and familiar objects relative to control animals when tested in later adulthood indicative of deficits in working memory.

The current behavioural findings extend our previous work using female rats (O'Shea et al., 2004), although some differences are noted. Previously we found impaired object recognition and decreased social interaction specific to adolescent, but not adult CP 55,940 exposure. We concluded that younger rats were more vulnerable to lasting behavioural

change induced by chronic cannabinoid exposure. Although the reasons for the differences between the present results and our previous work are not well understood, one possibility is that adult males may be more sensitive to chronic cannabinoid exposure than adult females. Sex-related differences in cognition and affect in general have been observed in humans (Halpern, 2000) as well as animals (Beatty, 1979), and structural and biochemical sex differences have also been demonstrated (Arnold & Gorski, 1984). Furthermore, previous work with rats (for review see Fernández-Ruiz et al., 1992; Moreno et al., 2003; Navarro et al., 1996) suggests that males may be more sensitive to chronic cannabinoid exposure. Studies with humans also support the existence of sex-related differences in cannabinoid effects. For example, a study by Pope and Yurgelun-Todd (1996) showed that cannabis-using males exhibited poorer performance on tests of cognition relative to females.

The memory deficits in rats treated with CP 55,940 at a perinatal age agree with previous animal research (i.e., cognitive deficits later in life as demonstrated in tasks assessing anxiety, memory and learning) (Gianutsos & Abbatiello, 1972; Kawash et al., 1980; Mereu et al., 2003). Further, these results mimic human studies showing an association between *in utero* exposure and later life cognitive deficits (i.e., memory and learning) (Fried et al., 2003; Richardson et al., 2002).

The results on adolescent CP 55,940-treated rats are in good agreement with previous research showing learning deficits in rats exposed to cannabinoids at this same age (30-40 days old) (Fehr et al., 1976; Fehr et al., 1978; Stiglick & Kalant, 1982a; Stiglick & Kalant, 1982b; Stiglick & Kalant,

1983). The present results also agree with the findings of a human study (Schwartz et al., 1989) showing that cannabis-using adolescents maintained working memory deficits following a 6 week drug-free period. Further, early versus late onset cannabis exposure has been compared at an adolescent age. One study (Ehrenreich et al., 1999) found attention deficits specific to early (before age 16) as opposed to late onset (16+) cannabis exposure. Pope et al. (2003) conducted a similar study comparing early (before age 17) versus late (17 years old+) onset cannabis exposure, and found IQ deficits associated with early onset. Further, these findings coincide with evidence that early onset (before age 17) cannabis exposure is associated with morphological brain change (Wilson et al., 2000).

The memory deficits in adult CP 55,940-treated rats are similar to attention deficits produced by THC at adult onset (Verrico, Jentsch, Roth, & Taylor, 2004). Male rats were treated with high doses of THC, and showed impaired performance on a serial reaction time task of attention when measured up to 14 days after the last drug administration. Conversely, a direct adolescent-adult comparison in male rats (Schneider & Koch, 2003) showed that adolescent, but not adult WIN 55,212-2-treated rats showed deficits in sensorimotor gating, object recognition, and performance in a progressive ratio task 10 days after the last drug exposure.

Two unusual findings in the object recognition experiment are worthy of comment. First, preference for the novel object relative to the familiar object during T2 was expected to decrease as the length of the delay was increased, but this did not occur. We have previously found a lack of effect of delay length on object recognition (O'Shea et al., 2004) and had assumed that the

delays used in our previous study (2 and 6 h) were too similar in duration. We therefore included a much longer delay (48 h) in the current study, but once again failed to find delay-dependency of object recognition. The possibility therefore remains that the range of delays used (2, 6, 48 h) is still too narrow to observe significant delay-dependency in our measures. The use of a shorter delay (e.g., 15 min) would clarify this point.

Second, locomotor activity during T2 of the object recognition test was lower in adult CP 55,940-treated rats relative to vehicle-treated rats, but only at the 2- and 6-h delays and not at the 48-h delay. This effect is unlikely due to drug residue as animals were tested following a 28-day drug free period. It is not clear why exposure to CP 55,940 should produce a long-lasting reduction in spontaneous locomotion, but at least one other study has reported similar findings in adult mice exposed to anandamide during the perinatal period (Fride & Mechoulam, 1996).

In conclusion, results from the present study suggest that cannabinoid exposure throughout all periods of life may compromise cognitive abilities that influence working memory. However, these findings alone are not sufficient to determine whether the residual working memory deficits are permanent, and future studies might also employ longer drug-free periods to that used in the current study. Nonetheless, there is growing evidence that cannabis exposure, from the perinatal period through to adulthood, may lead to lasting cognitive deficits.