CHAPTER 5: LASTING SOCIAL ANXIETY IN ADULT RATS TREATED WITH CP 55,940 AT PERINATAL, ADOLESCENT, AND EARLY ADULT AGES

Abstract

Although it is well known that a major side effect of acute cannabis intoxication is often anxiety, few studies have addressed possible lasting anxiogenic effects, particularly the effects on social anxiety. 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 35-day drug-free period, anxiety-like behaviour was assessed using a social interaction test. Reduced social interaction was evident in perinatal, adolescent, and adult CP 55,940-treated rats compared with controls. These results suggest that chronic exposure to a cannabinoid receptor agonist can lead to a lasting anxiogenesis in social interaction regardless of age at exposure.

5.1 Introduction

One of the most widely cited effects of acute cannabis intoxication in humans is the promotion of anxiety (Thomas, 1996). This finding is not surprising, considering many drug-using populations have been reported to exhibit higher levels of anxiety than the general population (Grenyer, Williams, Swift, & Neill, 1992; Meyer, 1986; Rounsaville et al., 1991). More specifically, panic-like symptoms are not uncommon to both acute and chronic cannabis use (Hall et al., 1994; Tournier, Sorbara, Gindre, Swendsen, & Verdoux, 2003). A recent cohort study (Patton et al., 2002) sought to determine whether cannabis use in adolescence predisposes to higher rates of depression and anxiety in young adulthood. Daily cannabis use in young females was associated with a fivefold increase in the odds of reporting a state of depression and anxiety. These results suggest that cannabis use in females in particular predicts later psychological disorders.

In animals, these same anxiety-like effects are found by administering THC, and other cannabinoids such as CBD (van Ree, Niesink, & Nir, 1984), HU-210 (Giuliani et al., 2000), and CP 55,940 (Arevalo, de Miguel, & Hernandez-Tristan, 2001). The amygdala is believed to play a major role in the development and expression of anxiety (Davis, 1992). A study by Onaivi et al. (1995) involved the direct injection of THC into the central nucleus of the amygdala of mice, resulting in an immediate anxiety-like 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 et al., 1997).

The majority of animal studies on anxiety-like behaviour are focused on the effects of current intoxication. For example, a study by van Ree et al. (1984) involved the administration of THC to rats, followed by assessment in the social interaction test. The high dose of THC (10 mg/kg) prevented most social interactions, whereas the low dose of THC (1 mg/kg) exerted selective and specific effects on social behaviour such as decreased grooming, crawling over/mounting, and aggressive behaviours (fighting, kicking, and biting).

A few studies have investigated residual anxiety-like effects. One study (Navarro et al., 1996) exposed rats to hashish extract (20 mg/kg THC) from GD 5 to PND 24. The behaviour of male and female rats was evaluated in adulthood (70 days old). In the social interaction test, the normal reduction in the time spent in active social interaction following exposure to a neophobic situation (high light levels) in controls did not occur in hashish-exposed males, thus demonstrating lower anxiety in drug-treated rats. These findings suggest that cannabinoid exposure is associated with decreased emotionality.

Another study (Giuliani et al., 2000) involved the administration of high doses of HU-210 (0.025-0.1 mg/kg) in adult (56-day old) rats. Behavioural testing in novel environments took place 1 h, 24 h, and 7 days after the last drug injection. Rats showed anxiety-like behaviour whilst intoxicated, and the persistence of enhanced anxiety in response to novel environments after the treatment was discontinued.

Another recent study (Schneider & Koch, 2004) assessed the effects of perinatal lesions (surgery on PND 7) of the mPFC on play behaviour, social behaviour, and self-grooming in adolescent (30-day old) male Wistar rats.

Additionally, the investigators sought to determine whether these lesions render the brain more vulnerable to cannabinoid exposure in later life. Thus, adolescent rats then received chronic treatment (25 days) with WIN 55,212-2 (1.2 mg/kg) or its vehicle starting at 40 days of age. During adulthood (80-day old) rats were again tested on previously assessed behaviours. Both lesions of the mPFC and adolescent WIN 55,212-2-exposure disrupted play fighting, social behaviour, and self-grooming.

There are more than 30 animal models of anxiety in existence, the vast majority behavioural in design (for review see Rodgers, Cao, Dalvi, & Holmes, 1997). These behavioural tasks can be "conditioned" or "unconditioned" models. Conditioned models commonly require extensive training periods, food or water deprivation, or sometimes electric shock. Unconditioned models rely on spontaneous behaviour alone and consequently can be considered to have a higher degree of ecological validity (Rodgers et al., 1997). One such test is the social interaction test developed more than 25 years ago (File & Hyde, 1978). It is one of the first unconditioned tests of anxiety in that it uses ethologically relevant sources of anxiety (social behaviour), and is devoid of food or water deprivation, electric shock, and extensive training (File & Seth, 2003).

The social interaction test is one of the most widely used animal models of anxiety (File, 1980). This test has been found to be sensitive to anxiety-increasing and anxiety-reducing drugs (File, 1980; File, Baldwin, Johnston, & Wilks, 1988), and has proven utility in screening new pharmacological agents (File, 1997). Most relevant to the current study, it has

also been used to assess the behavioural effects of CP 55,940 (Genn, Tucci, Marco, Viveros, & File, 2004).

This test allows the analysis of emotional reactivity in a neophobic situation and involves measuring the anxiety displayed by treatment-paired rats meeting for the first time. Social interaction typically decreases when rats are anxious. This test also utilises the sensitivity of rats to anxiogenic stimuli generated by a novel test arena and/or a brightly lit area (File, 1997).

Test arena conditions such as light and familiarity can be manipulated to achieve particular results in the social interaction test. 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. Barbiturates tend to increase social interaction in all conditions (i.e., an anxiolytic effect). These effects are best detected in conditions that generate low levels of interaction (e.g., high light, unfamiliar condition), whereas anxiety-like behaviour is better detected in the low light, familiar test arena condition (File, 1997). Commonly used social interaction indicators include sniffing, following, grooming, mounting, and crawling under or over the unfamiliar rat (File, 1980).

The clinical classification of anxiety (see Diagnostic and Statistical Manual of Mental Disorders: Forth Edition, Revised (DSM-IV-R) American Psychiatric Association, 2000) entails several subdivisions under the umbrella term "anxiety". These include generalised anxiety disorder (GAD), panic disorder (with or without agoraphobia), obsessive-compulsive disorder, social phobia, post-traumatic stress disorder, and simple phobias. This in turn raises the question as to whether animal models of anxiety are capable of measuring subtypes of anxiety disorders (File, 1992). It has been argued that the social

interaction test mimics the state of anxiety most similar to that experienced in GAD (File & Seth, 2003). However, behavioural and pharmacological evidence also suggests that this model is comparable to social anxiety in humans (Kantor et al., 2000).

So far, little research has been conducted on possible long-lasting (residual) anxiety following cannabinoid exposure. 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. Following a 35-day drug-free period, social anxiety was assessed in the social interaction test. Decreased social interaction (increased anxiety) was observed in adolescent but not adult CP 55,940-treated rats.

The purpose of the current study was to systematically assess perinatal (PND 4), adolescent (PND 30), and adult (PND 56) cannabis exposure onset on social interaction. It was expected that immature cannabinoid groups (i.e., the perinatal and adolescent drug-exposed groups) would show decreased social interaction relative to immature controls. In adult groups, it was also anticipated that the cannabinoid group could manifest a decrease in social interaction relative to adult controls.

5.2 Materials and Method

5.2.1 Subjects

As described previously (Chapter 4).

5.2.2 Drug Preparation and Administration

As described previously (Chapter 4).

5.2.3 Apparatus and Procedure

Following a 35-day drug-free period rats were tested in the social interaction test. At the onset of testing, perinatal groups were now 60 days old, adolescent groups were 86 days old, and adult groups were 112 days old. The experimental chamber was a rectangular box constructed of clear glass (620 x 300 x 360 mm), lit by a 60 W floor lamp located 1 m away from the box (Lux reading 062). Wood shavings were scattered on the floor of the chamber. Rats were habituated to the chamber on the day prior to testing for two non-consecutive 2-min periods. Testing began the next day. Each rat was paired with an age- and treatment-matched rat of approximately the same body weight for 10 min. There were six pairs for each treatment condition at each age group. Each trial was videotaped using a black and white CCD camera. An observer blind to the group allocations manually scored the video recorded trials using ODLog software.

Social behaviour for each member of pairs was examined. Scored behaviours included sniffing, following, grooming, mounting, and crawling under/over the stimulus rat.

5.3 Statistical Analysis

For each rat, the amount of time spent sniffing, following, grooming, mounting, and crawling under/over the other rat were summed to produce a single social interaction score. In addition, each of these individual social behaviours were analysed in isolation. Separate *t*-tests were used to compare treatments at each age group. A two-way ANOVA (age x treatment) was used to compare social interaction between perinatal, adolescent and

adult groups. Groups were then compared using *post hoc* Tukey tests where significant main effects were found.

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.

5.4 Results

An independent samples *t*-test used to compare the overall social interaction between perinatal groups revealed that CP 55,940-treated rats showed significantly less social interaction than the vehicle-treated group [$t(22)=3.34$, $p<0.01$]. (See Figure 5.1; for statistics outputs see Appendix A29 and for data see Appendix B15).

Separate *t*-tests comparing each social behaviour between perinatal groups revealed that CP 55,940-treated rats showed significantly lower levels of sniffing [$t(22)=2.45$, $p<0.05$], following [$t(22)=2.72$, $p<0.05$], and crawling under/over the unfamiliar rat [$t(22)=2.66$ $p<0.05$] compared with vehicle controls. (See Figure 5.2; for statistics outputs see Appendix A32 and for data see Appendix B18).

An independent samples *t*-test used to compare the overall social interaction between adolescent groups revealed that CP 55,940-treated rats showed significantly lower social interaction than the vehicle-treated group [$t(22)=3.29$, $p<0.01$]. (See Figure 5.3; for statistics outputs see Appendix A30 and for data see Appendix B16).

Separate *t*-tests comparing each social behaviour between adolescent groups revealed that CP 55,940-treated rats showed significantly lower levels of sniffing [$t(22)=3.52$, $p<0.01$] and following the unfamiliar rat [$t(22)=2.32$, $p<0.05$] compared with controls. (See Figure 5.4; for statistics outputs see Appendix A33 and for data see Appendix B19).

Likewise, adult CP 55,940-treated rats showed less overall social interaction than vehicle controls [$t(22)=3.61$, $p<0.01$]. (See Figure 5.5; for statistics outputs see Appendix A31 and for data see Appendix B17).

Separate *t*-tests comparing each social behaviour between adult groups revealed that CP 55,940-treated rats showed significantly lower levels of sniffing [$t(22)=3.91$, $p<0.01$] in comparison to controls. (See Figure 5.6; for statistics outputs see Appendix A34 and for data see Appendix B20).

A two-way ANOVA (age x treatment) comparing social interaction between perinatal, adolescent, and adult groups revealed a significant main effect of age [$F(2,66)=5.12$, $p<0.01$], and a significant main effect of treatment [$F(1,66)=34.45$, $p<0.001$]. The age x treatment interaction was not significant [$F(2,66)<1.0$]. Tukey *post hoc* tests comparing ages revealed that adolescent rats showed significantly lower social interaction than adult rats. See Figure 5.7.

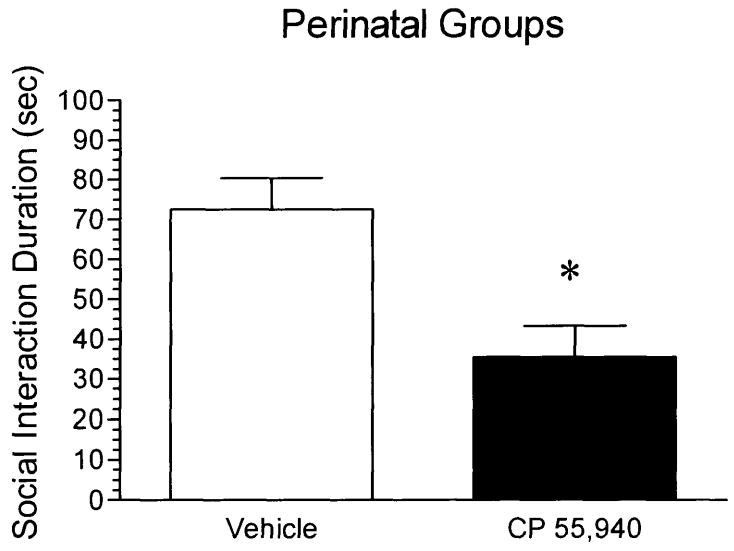

Figure 5.1 Total mean time (sec) spent in social interaction for perinatal vehicle- (n=12) and CP 55,940-treated rats (n=12). *CP 55,940 rats spent significantly less time in total social interaction.

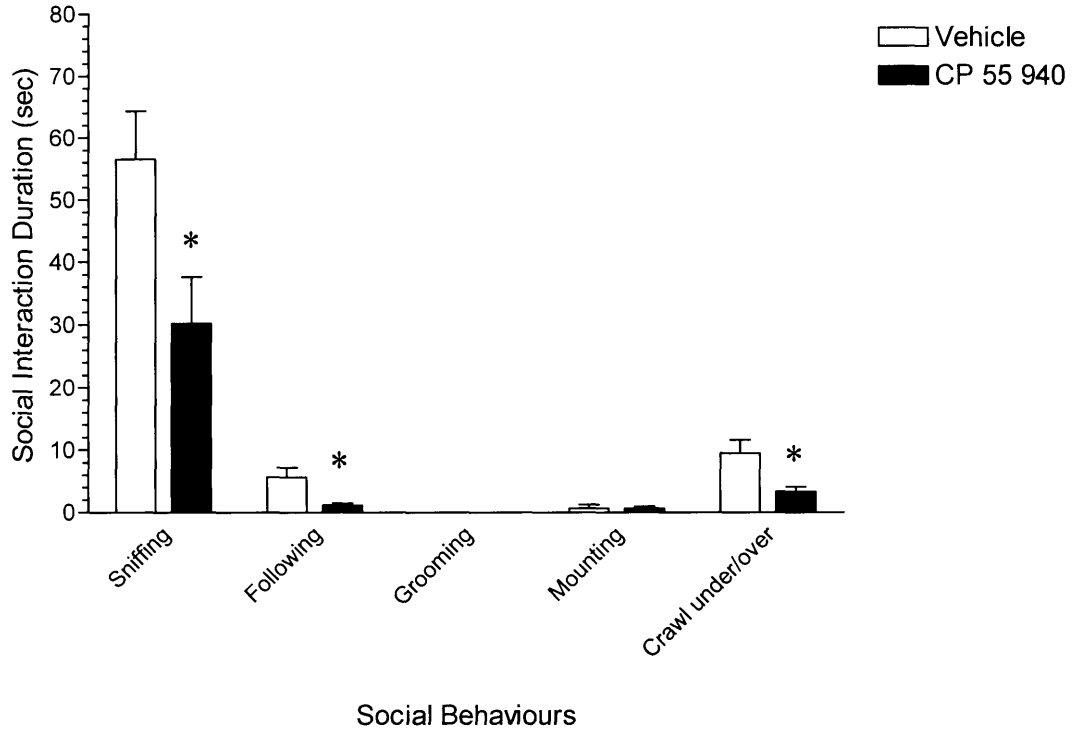

Figure 5.2 Time (sec) spent exhibiting individual social interaction behaviours for perinatal vehicle- (n=12) and CP 55,940-treated rats (n=12). *CP 55,940 rats spent significantly less time exhibiting social behaviour.

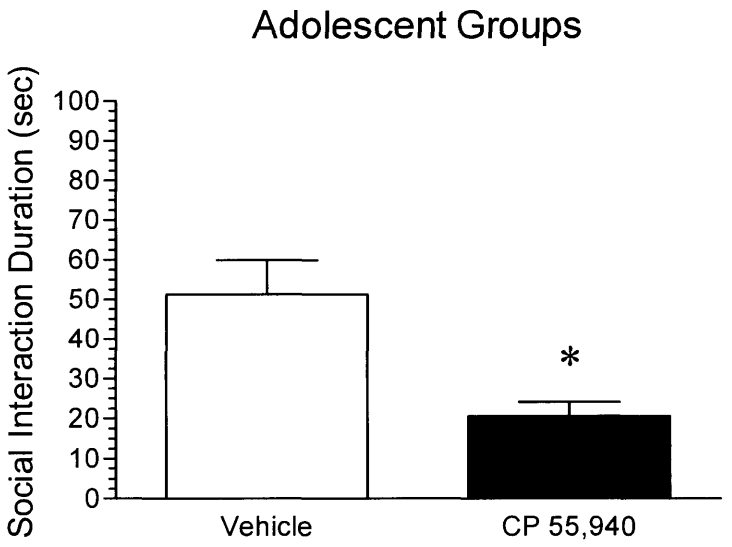

Figure 5.3 Total mean time (sec) spent in social interaction for adolescent vehicle- (n=12) and CP 55,940-treated rats (n=12). *CP 55,940 rats spent significantly less time in total social interaction.

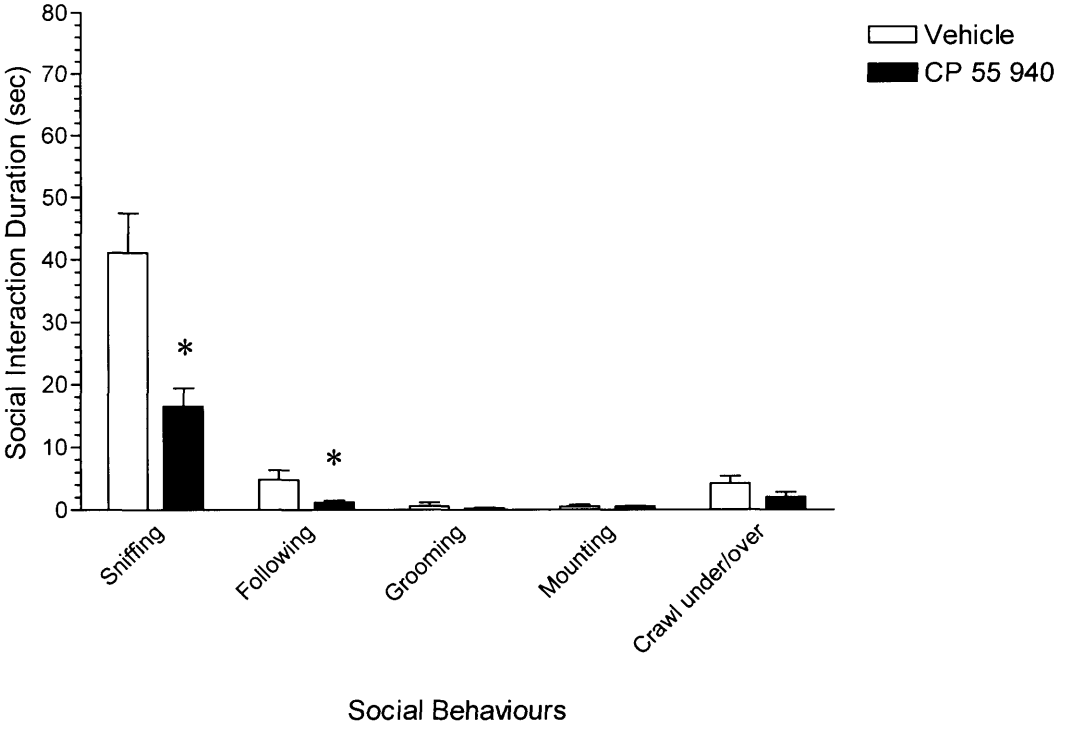

Figure 5.4 Time (sec) spent exhibiting individual social interaction behaviours for adolescent vehicle- (n=12) and CP 55,940-treated rats (n=12). *CP 55,940 rats spent significantly less time exhibiting social behaviour.

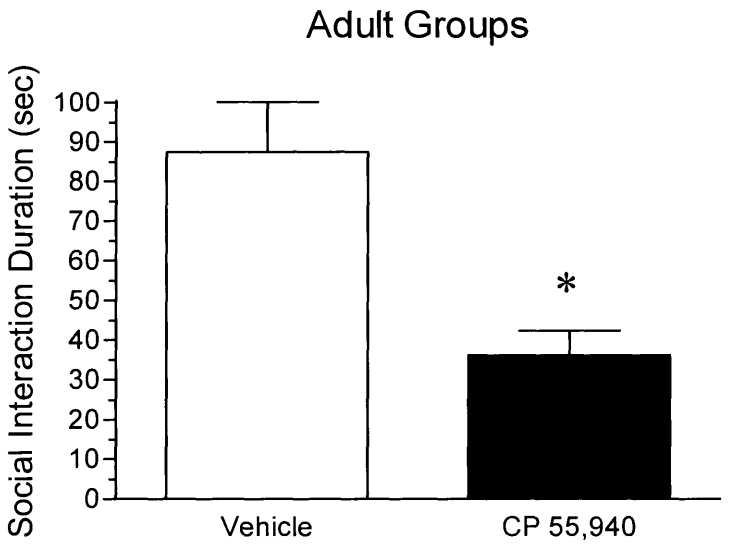

Figure 5.5 Total mean time (sec) spent in social interaction for adult vehicle- (n=12) and CP 55,940-treated rats (n=12). *CP 55,940 rats spent significantly less time in total social interaction.

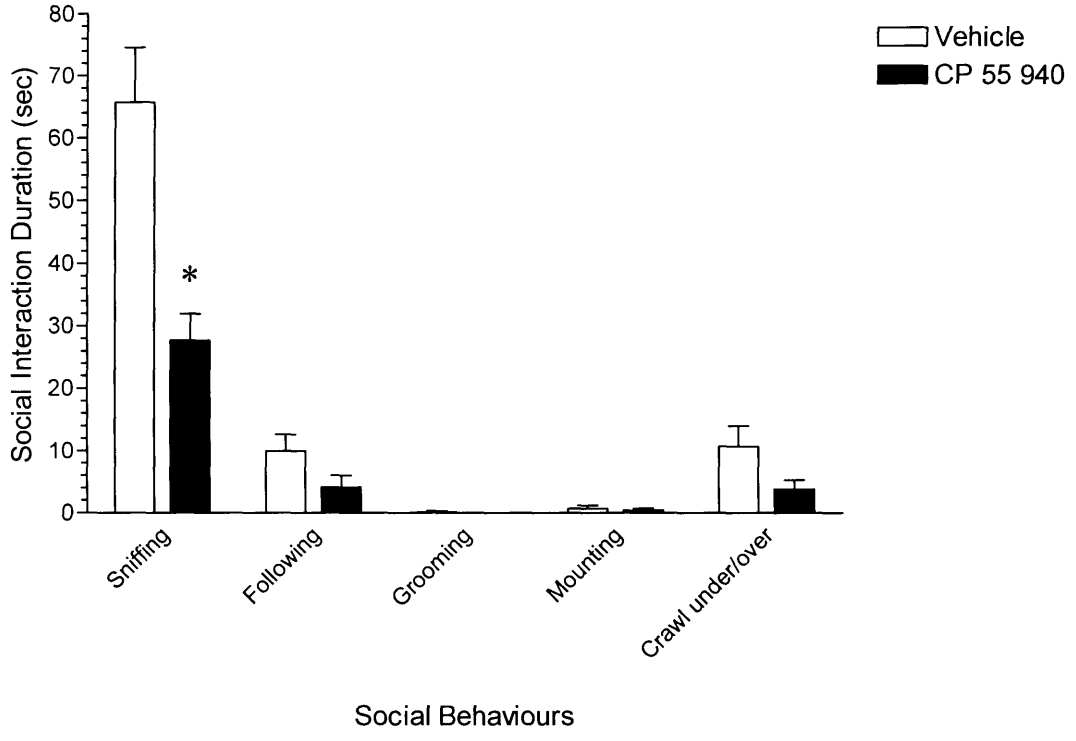

Figure 5.6 Time (sec) spent exhibiting individual social interaction behaviours for adult vehicle- (n=12) and CP 55,940-treated rats (n=12). *CP 55,940 rats spent significantly less time exhibiting social behaviour.

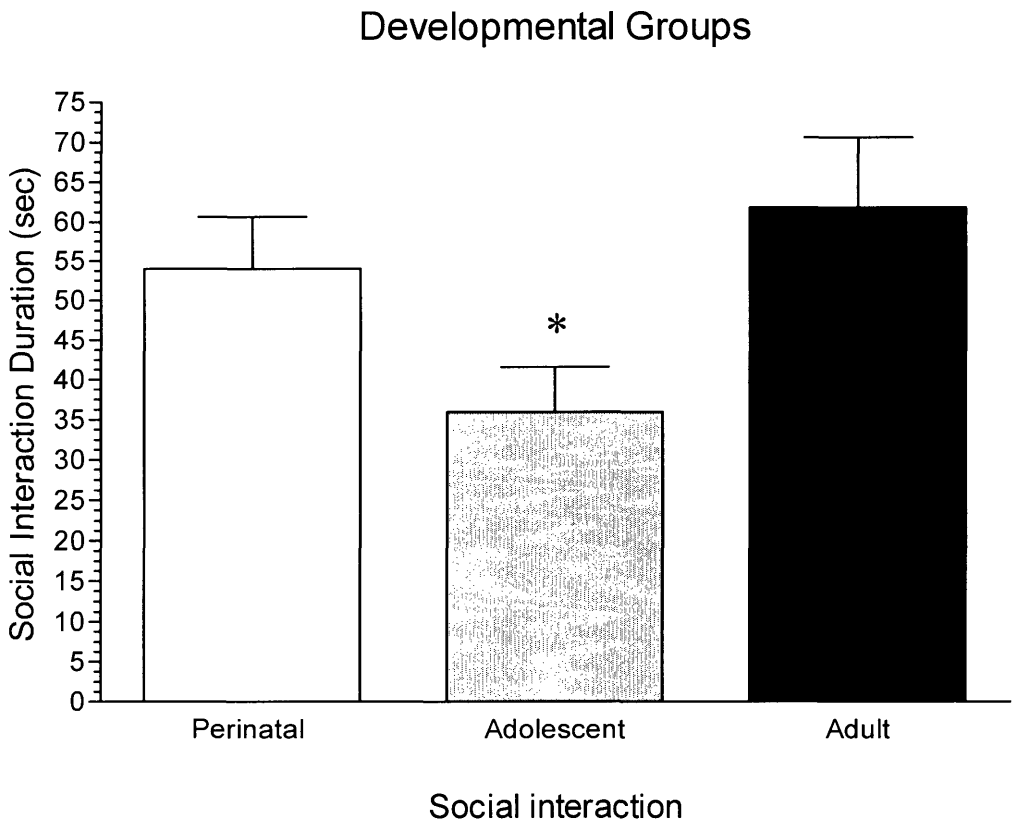

Figure 5.7 Time (sec) spent in social interaction for perinatal, adolescent, and adult age groups. CP 55,940 and vehicle data averaged at each developmental age. *Adolescent rats showed significantly lower social interaction than adult rats.

5.5 Discussion

It was hypothesised that immature cannabinoid groups (i.e., the perinatal and adolescent drug-exposed groups) would show decreased social interaction (social anxiety) relative to immature vehicle controls. In adult groups, it was also anticipated that the cannabinoid group could manifest a decrease in social interaction relative to adult controls. The results showed that rats treated with CP 55,940 in perinatal, adolescent, and early adult period exhibited lower social interaction than age-matched controls. Overall, these findings suggest that lasting anxiogenesis is associated with cannabinoid exposure regardless of age at onset.

A previous study by the author (O'Shea et al., 2004) also showed decreased social interaction in female rats exposed to CP 55,940, but these findings were specific to rats treated with CP 55,940 in adolescence. Combined, these results provide strong evidence that cannabinoid exposure, despite discontinuation, might lead to lasting effects on anxiety-like behaviour. However, it may be the case that these effects more readily occur in male rats regardless of age. At the time of the previous study, we had assumed that the differing findings between immature (adolescent) and mature (adult) rats might have supported lasting behavioural change as a consequence of age at exposure. The current results may also support sex-related differences in lasting cannabinoid effects.

Further, CP 55,940-treated males regardless of age (i.e. perinatal, adolescent, adult) showed deficits in an object recognition task assessing working memory days prior to the current study (Chapter 4). The previous

adolescent-adult comparison in female rats (O'Shea et al., 2004), however, showed working memory deficits specific to adolescent exposure.

There are a few possible explanations for the current findings. One explanation is that male rats may incur greater residual deficits (i.e., anxiety and memory) as a consequence of past cannabis exposure, regardless of age at exposure. There is some evidence that male rats are more sensitive to the neurobehavioural effects of cannabinoids. A study by Navarro et al. (1996) found that perinatal hashish exposure in males, but not females, led to marked changes in the behavioural patterns executed in a sociosexual approach behaviour test in adulthood. In addition, these behavioural changes were accompanied by decreased mesolimbic dopaminergic activity. Another study (Fernández-Ruiz et al., 1992) addressing perinatal cannabinoid effects on dopaminergic neurons found that these effects differed according to sex and the specific pathways studied, with the most profound and consistent alterations in males.

Another explanation may relate to the ethological validity of the social interaction test itself. Although the test has been validated using adult male (File & Seth, 2003) and female rats (Kellogg, Awatramani, & Piekut, 1998), as well as adolescent male rats (File & Tucker, 1984a; File & Tucker, 1984b), this test has primarily been utilised with adult males. Therefore, an important sex-related difference between male and female rats might be that female rats do not increase social interaction as markedly in response to increasing familiarity with the test arena (Johnston & File, 1991). It is possible that social interaction serves a different function in male and female rats and caution should be exercised when interpreting results in females (File & Seth, 2003).

Further, it has been suggested that male gonadal function (testosterone) affects the development of specific neural systems underlying social interaction (Primus & Kellogg, 1990).

Another explanation might be differing sex-related responses to environmental manipulations. In the current experiment, the low light, familiar test arena condition was used. Social interaction is highest when rats are tested in this condition, and it is said to best detect anxiety-like behaviour (File & Seth, 2003). However, it been suggested that female rats do not increase social interaction as markedly in response to increasing familiarity with the test arena (Johnston & File, 1991). Further, Kellogg, Primus, and Bitran (1991) noted that adult (60-90 days old) rats exposed to diazepam (an anxiolytic drug) in perinatal life show sex-dependent behaviour in the social interaction test. Adult male rats exhibited increased social interaction in the unfamiliar environment, and decreased social interaction in a familiar environment, whereas the social interaction of female rats was comparable to control males.

In the current study it is interesting to note that baseline social interaction in adolescents is lower than that of perinatal and adult rats. This is at odds with the finding that older rats typically spend less time in social interaction than rats of a younger age (Salchner, Lubec, & Singewald, 2004). This result was also found in the adolescent female rats previously (O'Shea et al., 2004). We had attributed this difference to either an age-related difference in the response to mild chronic injection stress experienced during the drug administration phase (Jaskiw, Karoum, & Weinberger, 1990), or sex-related behavioural differences in this test (Navarro et al., 1994; Navarro et

al., 1996). This second explanation however, is unlikely. It is not known whether this reduction in social interaction is related to locomotor activity, as this was not measured in the social interaction test. Locomotor activity in these same rats was however measured in the object recognition task (Chapter 4). No significant differences in locomotor activity were apparent, except that CP 55,940-treated adult rats showed some evidence of lower locomotor activity. It is not known why exposure to CP 55,940 should produce a long-lasting reduction in spontaneous locomotion, but similar long-lasting locomotor deficits have been reported previously (Fride & Mechoulam, 1996).

Looking at the results of perinatal, adolescent, and adult groups separately, the anxiety displayed by perinatal CP 55,940-treated rats is in disagreement with previous studies. One study (Rubio et al., 1995) suggested that perinatal THC exposure was associated with reduced anxiety in adulthood. Male and female rats exposed to THC on GD 5 to PND 24 exhibited increases in rearing, grooming, and sniffing in adulthood when assessed on motor behaviours. Additionally, female THC-treated rats showed greater locomotor activity than controls, whereas THC-exposed males exhibited increased exploratory behaviour in a plus-maze paradigm. Another study in the same laboratory (Navarro et al., 1996) showed that perinatal THC exposure was associated with increased social interaction in adulthood, suggesting that male THC-treated rats showed less anxiety than controls.

Similarly, the results in adolescent CP 55,940-treated rats disagree with a previous study involving adolescent onset CP 55,940 exposure followed by behavioural testing in adulthood (Biscaia et al., 2003). This study utilised a variety of different stress conditions (illumination) in varied

paradigms (holeboard testing, open field testing and an elevated plus-maze) and found less emotionality/anxiety in rats treated with cannabinoids at adolescent onset.

The present results on adult rats agree with a previous study that examined the behaviour of adult rats 7 days after HU-210 exposure (Giuliani et al., 2000). At sub-chronic high doses of HU-210, rats continued to exhibit anxiety-like behaviour thought to reflect the persistence of an enhanced emotional response to novel environments even after treatment was discontinued.

The current results combined illustrate that more systematic testing of residual cannabinoid effects in both male and female rats at key developmental ages is needed to further validate whether the social interaction test is a suitable animal model of anxiety for animals of different age and sex. Systematic testing is also warranted using other animal models of anxiety to detect both sex-related differences and developmental effects. At present, the current results suggest that males are most at risk of lasting anxiogenesis due to cannabinoid exposure regardless of age at cannabis initiation.

CHAPTER 6: AGE-RELATED REDUCTIONS IN AGGRESSION IN CP 55,940-TREATED RATS

Abstract

Little is presently known about the putative link between cannabis use and aggressive behaviour, particularly whether cannabinoids facilitate or suppress aggression long after drug-exposure has ceased. 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 35-day drug-free period, social behaviour was assessed, inclusive of aggressive indices. The results showed that perinatal CP 55,940-treated rats and controls did not differ on aggressive behaviours. However, adolescent and adult CP 55,940-treated treated rats exhibited reduced aggressive behaviours in comparison with controls. These results suggest that chronic exposure to a cannabinoid receptor agonist might lead to a small, but lasting, decrease in aggression.

6.1 Introduction

Cannabis is one of the most popularly used recreational drugs worldwide. This applies to all countries in the Oceania region, most countries in Western Europe and North America, nearly all countries in Africa, and the majority of countries in Asia (United Nations Office on Drugs and Crime, 2004). Despite the prevalent use of cannabis, little is known about whether cannabis use is associated with aggression. In fact, despite the research focused on this investigation, it remains the case that aggression is one of the most controversial and contradictory behaviours associated with cannabis exposure (Mechoulam, 2002).

In animals, acute cannabinoid exposure typically suppresses, or does not alter aggressive behaviour (for review see Miczek, 1999). For example, a study by van Ree et al. (1984) involved the administration of low (1 mg/kg) or high doses (10 mg/kg) of THC or CBD (2 and 20 mg/kg) to rats socially isolated for 7 days. Rats were then assessed in the social interaction test. The high dose of THC prevented nearly all social interactions. The low dose decreased both non-aggressive (i.e., crawling over/mounting, and grooming) and aggressive behaviours (i.e., fighting, kicking, and biting). Both low and high doses of CBD did not affect social interaction.

In humans, there is a lack of association between acute cannabis intoxication and aggression (Dhossche, 1999; Taylor et al., 1976). For example, a human study by Tart (1970) found that 77% percent of cannabis users did not engage in antisocial acts whilst intoxicated, 22% rarely engaged in antisocial acts whilst intoxicated, and 1% sometimes committed anti-social acts whilst intoxicated. Conversely, Niveau and Dang (2003) suggested that

cannabis use leads to violent behaviour, as the 12 violent offenders involved in this study were acutely intoxicated by cannabis at the time of committing violent acts. Subsequently, all offenders were judged by the court to be partially or totally non-responsible for their violent behaviour. However, 5 participants in this study had a pre-existing psychiatric history, and another 5 had a personality disorder.

In animals, chronic cannabinoid exposure has been associated with aggressive behaviour. For example, a study by Luthra, Rosenkrantz, Heyman, and Braude (1975) exposed rats to THC for up to 6 months, followed by a 30-day drug-free period. During drug administration, aggressive behaviour and irritability became apparent on days 10-20, although fighting behaviour peaked on days 20-100 at the highest dose of THC. Aggressive behaviour had dissipated following the 30-day drug-free period but some neurochemical alterations remained apparent.

Some human studies, however, show no association between chronic cannabis use and aggressive or criminal acts. A study by Soueif (1971) compared the criminal records of 553 prisoners incarcerated for cannabis-related crimes to 458 controls arrested on other charges. Cannabis users were defined as having used cannabis at least once per month in the year preceding incarceration. Fewer cannabis users had criminal records prior to their arrest (5.7%) compared to controls (13.5%). Furthermore, controls had committed a higher number of crimes per person (5.3%) compared with cannabis users (4.5%). Interestingly, 56% of non-cannabis using prisoners believed that cannabis users engaged in more criminal acts than non-users of the drug.

In animals, cannabis withdrawal has been associated with aggression. Cutler, Mackintosh, and Chance (1975b) fed cannabis to mice for 2 weeks and assessed social behaviour during and after drug-administration. During treatment, dominant cannabis-treated males exhibited decreased non-social behaviour (such as exploration and self-grooming), increases in flight (e.g., evading, retreating, fleeing), social investigation (e.g., approaching or grooming other mouse), and sexual behaviours (e.g., mounting). Following a 1-week drug-free period, these rats showed a significant increase in aggressive behaviours (e.g., biting, attacking, chasing, and offensive upright), which may have been due to drug withdrawal.

Some human studies also provide evidence of a cannabis withdrawal syndrome. A study by Kouri, Pope, and Lukas (1999) investigated aggressive behaviours in long-term cannabis users following abstinence. Chronic users (equivalent of 14 years daily use) were studied on day 0, 1, 3, 7 and 28 of a detoxification period. The highest levels of aggression were observed on days 3 and 7 of abstinence, although these behaviours normalised when measured at day 28. Similarly, another study (Budney, Hughes, Moore, & Novy, 2001) involved the observation of cannabis users over a 16-day period. On days 1-5 users smoked cannabis as usual. On days 6-8 users abstained from cannabis use, returned to cannabis consumption on days 9-13, and again abstained on days 14-16. Aggressiveness, anger, irritability, and restlessness were observed during abstinence, however this likely reflected drug withdrawal and dependence, rather than representing a correlation between cannabis use and aggression as such.

Several reports indicate that aggressive behaviour in rats is provoked by pairing drug exposure with aversive stimuli (for review see Miczek, 1999). One study (Alves, Goyos, & Carlini, 1973) administered THC (5–40 mg/kg) and several cannabis extracts to 96-h REM (rapid eye movement) sleep deprived rats. Dose-dependent aggressive behaviour (biting, standing on hind legs, upright posture) and irritability were observable for at least 4 h following drug administration. Conversely, lysergic acid diethylamide (LSD), mescaline, *d*-amphetamine, and ethanol failed to elicit these same effects. However, another study indicated that a range of pharmacological drugs, as well as cannabis, increased aggression in REM sleep deprived rats (Carlini, Lindsey, & Tufik, 1971).

A study by Carder and Olson (1972) paired THC exposure with electric foot shock. Rats treated with doses of 0.25 and 0.50 mg/kg of THC fought more than controls. A dose of 0.12 mg/kg was ineffective, while doses of 1 mg/kg and 2 mg/kg suppressed fighting. When animals habituated to the test situation, no change in aggression was observed. This study illustrates that stress, dose, and test condition novelty might mediate THC-induced aggression.

Cannabinoid dose in particular plays a diverse role in aggressive behaviour. In animals, it has been suggested that low cannabinoid doses reduce aggression (Cherek, Thompson, & Kelly, 1980) and high doses increase aggression (Alves et al., 1973). In humans, the converse has been suggested. That is, low doses slightly increase aggression, and moderate and high doses can reduce aggressive behaviour (Myerscough & Taylor, 1986).

Similarly, some studies have found aggression related to both dose and the vehicle used to transport THC. For instance, a study by Dorr and Steinberg (1976) compared two vehicles for THC (1.25-20 mg/kg) comprising either 1% Tween 80-saline, or a combination of propylene glycol, Tween 80, and saline. In general, social behaviour decreased as THC dose increased. Further, low doses of THC in the propylene glycol vehicle decreased social behaviour (including aggression), and low doses of THC in the vehicle lacking propylene glycol sometimes stimulated aggressive behaviour. A dose of 2.5 mg/kg THC, in either vehicle markedly increased both aggression and flight acts.

Social conditions can also mediate aggressive behaviour in cannabinoid-treated animals. One study (Sulcova, Mechoulam, & Fride, 1998) administered ANA to group- or single-housed mice and assessed antagonistic behaviour. Mice were paired with an age- and sex-matched opponent (group- or single-housed). ANA at doses of 0.01 or 0.1 mg/kg did not affect behaviour in single-housed mice. The highest dose (10 mg/kg) decreased aggressive behaviour, increased defence-escape behaviour (timidity), and decreased locomotor activity. In group-housed mice, the lowest dose (0.01 mg/kg) stimulated aggression, and the highest dose (10 mg/kg) inhibited social behaviour and locomotor activity, without stimulating either timid or aggressive behaviour.

Another study (Cutler & Mackintosh, 1975a) devoid of overt stressors assessed the social behaviour of rats and mice following an acute THC (5 mg/kg) dose. THC-treated animals were paired with vehicle controls and 50 to 60 elements of social behaviour were assessed. THC-treated rats and

mice showed a significant decrease in non-social, social, and sexual activity as well as locomotor activity. In addition, flight was significantly increased in mice. Overall, the results showed that THC failed to elicit aggressive behaviour in both the rat and mouse.

While factors such as acute exposure (intoxication), chronic exposure (repeated exposure), and withdrawal (abstinence), aversive stimuli, dose, and social conditions appear to influence whether an aggressive response is inhibited or displayed, a particularly important consideration in animal models is whether aggression represents an acute effect of cannabis for the period of intoxication alone, irritability (stress) due to repeated cannabinoid injections, or withdrawal effects (Fehr et al., 1978).

One study (Fehr et al., 1978) eliminated these variables by assessing aggression after a drug-free period. Cannabis extract (20 mg/kg) or ethanol (6 g/kg) was administered to rats for a period of 6 months. Muricidal behaviour was assessed following a 30- or 60-day drug-free period. Both cannabis- and ethanol-treated rats exhibited higher levels of muricidal behaviour in comparison to controls 1 and 2 months after the last drug administration. Accompanying these behaviours were irregular spike-like waves in electrical recordings from the dorsal hippocampus.

The present study expands on the study by Fehr et al. (1978). Male Wistar rats were exposed to CP 55,940 on PND 4, PND 30, or PND 56, corresponding to perinatal, adolescent, and early adult developmental periods, respectively. CP 55,940 exposure spanned 21 days followed by a 35-day drug-free period, prior to assessment in the social interaction test (Chapter 5). It was expected that immature cannabinoid groups (i.e., the

perinatal and adolescent drug-exposed groups) might show increased aggressive behaviours in a social interaction test relative to immature controls. In adult groups, it was also anticipated that the cannabinoid group could manifest greater aggressive behaviour relative to adult controls.

The social interaction test (File & Hyde, 1978) involves the analysis of social behaviour in treatment-paired rats meeting for the first time. Aggressive behaviours include kicking, jumping on, and wrestling/boxing with an unfamiliar rat (File & Hyde, 1978).

6.2 Materials and Method

6.2.1 Subjects

As described previously (Chapter 4).

6.2.2 Drug Preparation and Administration

As described previously (Chapter 4).

6.2.3 Apparatus and Procedure

Following a 35-day drug-free period rats were tested in the social interaction test. See Chapter 5 for description. Aggressive behaviours for each member of pairs were examined. These behaviours included kicking, jumping on, and wrestling/boxing with the age- and treatment matched rat.

6.3 Statistical Analysis

Individual aggressive behaviours were analysed (kicking, jumping on, and wrestling/boxing with the stimulus rat). Separate *t*-tests were used to compare treatments at each age group. A two-way ANOVA (age x treatment) was used to compare the effect of treatment on aggressive behaviour across perinatal, adolescent and adult groups. Groups were then compared using *post hoc* Tukey tests where significant main effects were found.

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 ANOVAs were conducted using SPSS 12.0.1 for Windows.

6.4 Results

Perinatal CP 55,940-treated rats did not differ significantly from controls on behaviours such as kicking [no *t*-tests result as means=0], jumping on the unfamiliar rat [$t(22)=1.30$, $p>0.05$], or wrestling/boxing [$t(22)=1.66$, $p>0.05$]. (See Figure 6.1; for statistics outputs see Appendix A32 and for data see Appendix B18).

Adolescent CP 55,940-treated rats did not differ from controls on behaviours such as kicking [$t(22)<1.0$], and wrestling/boxing [$t(22)<1.0$]. However, CP 55,940-treated rats showed lower levels of jumping on the unfamiliar rat [$t(22)=2.68$, $p<0.05$]. (See Figure 6.2; for statistics outputs see Appendix A33 and for data see Appendix B19).

Adult CP 55,940-treated rats did not differ from controls on behaviours such as kicking [no *t*-tests result as means=0] and wrestling/boxing [$t(22)=1.50$, $p>0.05$], but showed significantly lower levels of jumping on the unfamiliar rat [$t(22)=2.82$, $p<0.05$]. (See Figure 6.3; for statistics outputs see Appendix A34 and for data see Appendix B20).

A two-way ANOVA (age x treatment) comparing aggressive social behaviours between perinatal, adolescent, and adult groups showed a significant main effect of age [$F(2,66)=20.85$, $p<0.001$], a significant main effect of treatment [$F(1,66)=8.61$, $p<0.01$], and a significant age x treatment

interaction [F(2,66)=4.37, $p<0.05$]. As a main effect of age was observed, *post hoc* Tukey tests revealed that aggressive social behaviour differed significantly between age groups. See Figure 6.4.

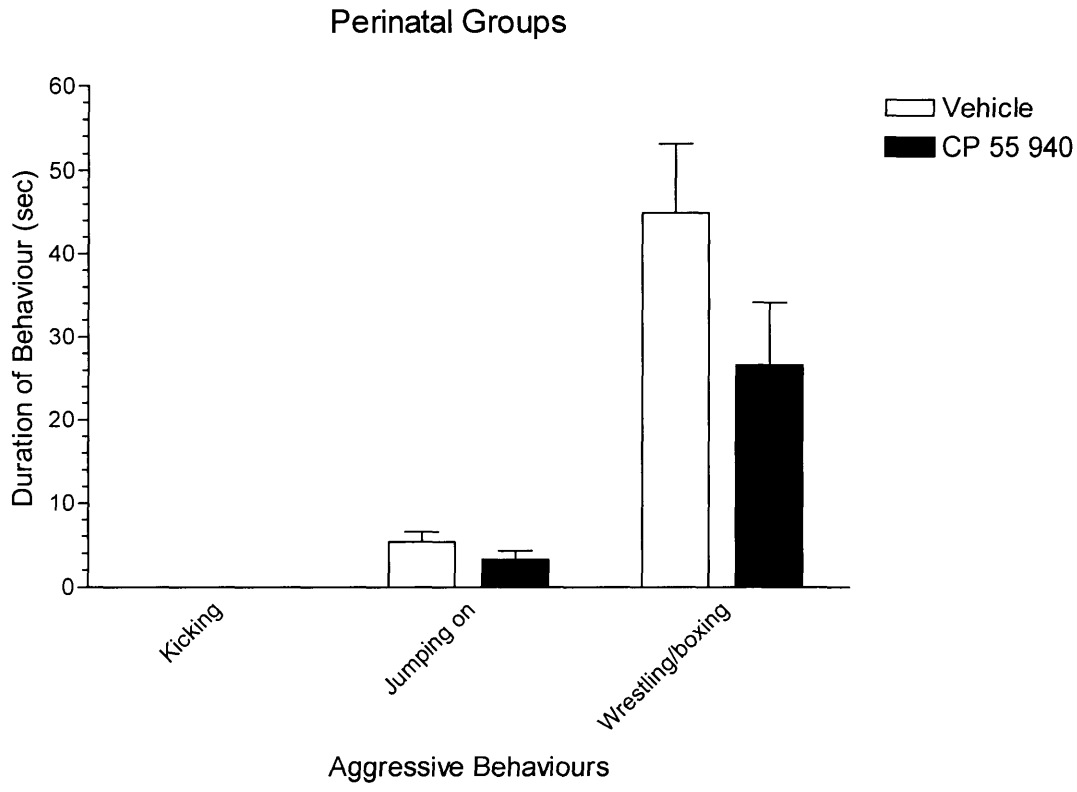

Figure 6.1 Time (sec) spent exhibiting aggressive behaviours for perinatal vehicle- (n=12) and CP 55,940-treated rats (n=12). Aggressive behaviours were classified as kicking, jumping on, and wrestling or boxing with the unfamiliar rat.

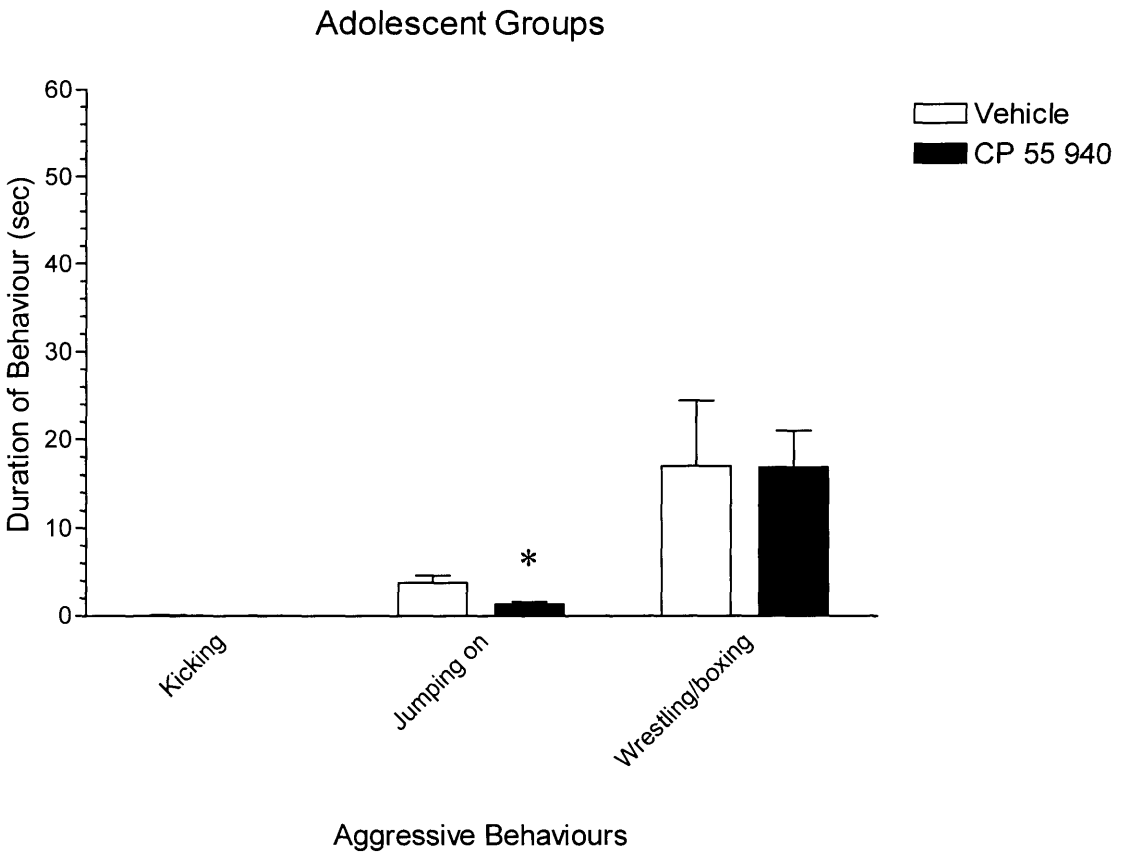

Figure 6.2 Time (sec) spent exhibiting aggressive behaviours for adolescent vehicle- (n=12) and CP 55,940-treated rats (n=12). Aggressive behaviours were classified as kicking, jumping on, and wrestling or boxing with the unfamiliar rat. *CP 55,940 rats spent significantly less time exhibiting aggressive behaviour.

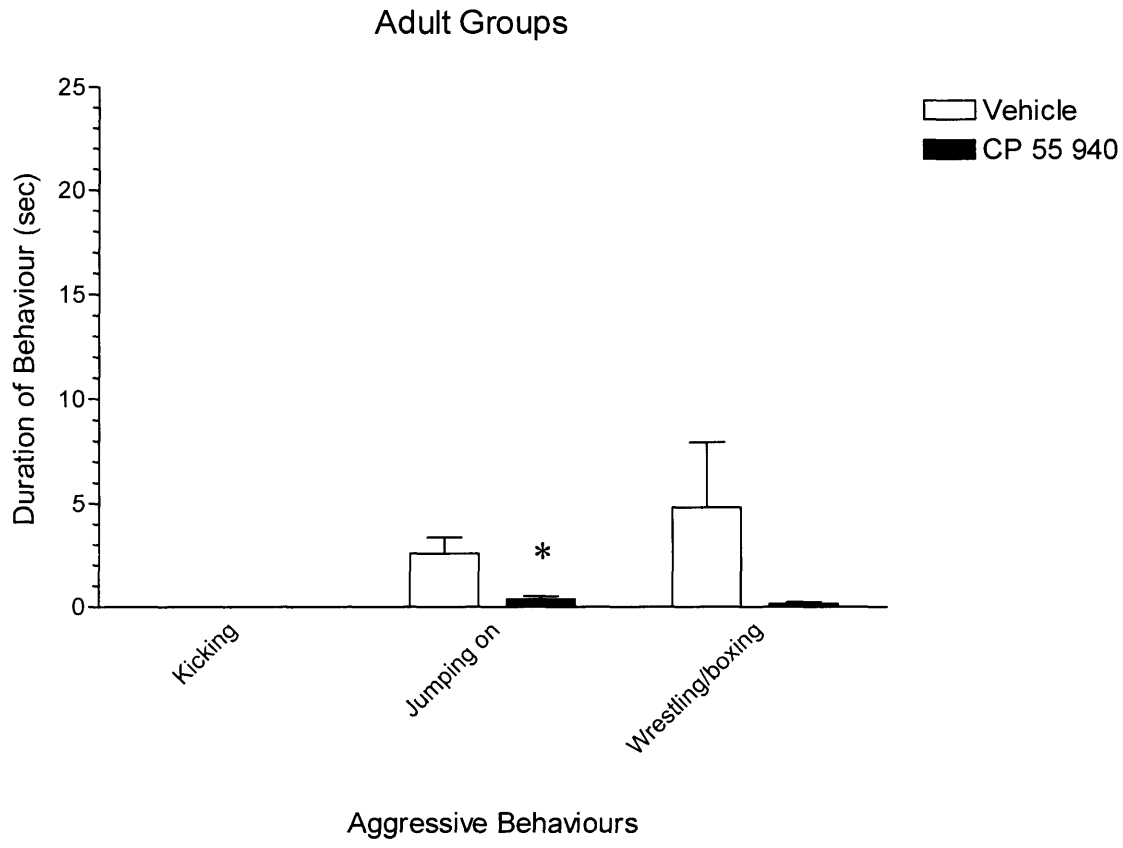

Figure 6.3 Time (sec) spent exhibiting aggressive behaviours for adult vehicle- (n=12) and CP 55,940-treated rats (n=12). Aggressive behaviours were classified as kicking, jumping on, and wrestling or boxing with the unfamiliar rat. *CP 55,940 rats spent significantly less time exhibiting aggressive behaviour.

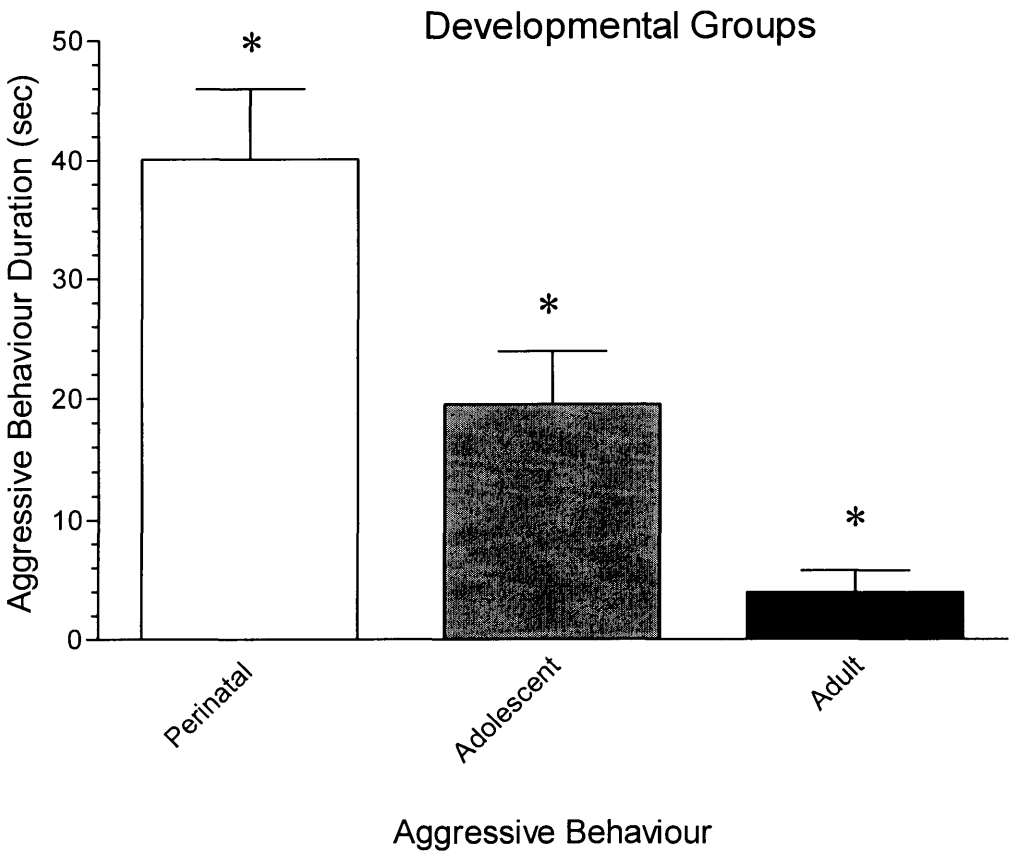

Figure 6.4 Time (sec) spent exhibiting aggressive behaviours for perinatal, adolescent, and adult age groups. CP 55,940 and vehicle data averaged at each developmental age. *Aggressive behaviour between all developmental age groups differed significantly.

6.5 Discussion

The current study assessed aggressive behaviours in rats treated with CP 55,940 at perinatal, adolescent, and adult ages. As with a previous study (Fehr et al., 1978), it was anticipated that immature cannabinoid groups (i.e., the perinatal and adolescent CP 55,940-exposed groups) might show increased aggressive behaviours in the social interaction test relative to immature controls. In adult groups, it was also anticipated that the cannabinoid group could manifest greater aggressive behaviour relative to adult controls.

The results showed that perinatal CP 55,940-treated rats and controls did not differ on aggressive behaviours. Adolescent and adult cannabinoid-treated rats however, exhibited marginally lower (albeit significant) aggressive behaviours in comparison with controls. These results suggest that cannabinoid exposure, despite cessation, might lead to a small decrease in aggression in later life. The current results are incongruent with cannabis-induced aggressive behaviour in Wistar rats following a protracted drug-free period (Fehr et al., 1978).

These same CP 55,940-treated rats exhibited reduced non-aggressive social behaviours (increased social anxiety) at all ages (Chapter 5). High social anxiety and low aggression has been observed in the social interaction test previously (Kantor et al., 2000). Further, the current results are similar to previous findings. One study (van Ree et al., 1984), involved the acute administration of THC or CBD followed by the assessment of social interaction. The overall results of this study suggested that the high dose of THC (10 mg/kg) prevented most social interactions, whereas the low dose of

THC (1 mg/kg) exerted selective and specific effects on rat social behaviour, such as decreased grooming, crawling over/mounting, and aggressive behaviours (fighting, kicking, and biting).

Similarly, another study (Cutler & Mackintosh, 1984) involved the administration of either cannabis extract or THC (4-100 mg/kg) in male mice. Shortly after, these rats were paired with vehicle-treated males and control females. Flight behaviours such as flagging, evading, retreating and fleeing were increased. Further, immobility was increased by cannabis extract in male-male encounters (50 and 100 mg/kg), male-female encounters (25 mg/kg); and by THC (5 mg/kg). Non-social behaviours such as scanning, washing and self-grooming, and some elements of social investigation, most markedly sexual behaviour in females, were also reduced.

Another study (Sieber, Frischknecht, & Waser, 1980a) involved both acute and chronic hashish exposure (THC 20 mg/kg) in adult mice followed by the assessment of social interaction. Acute hashish administration suppressed social interaction. Following chronic exposure, hashish-males exhibited an increase in non-social activities (such as self-grooming) as well as submissive behaviour and flight (e.g., defensive upright, retreat etc). Social investigation (e.g., follow, groom other partner) was less frequent, and sexual and aggressive behaviour were not altered by hashish exposure.

These same authors (Sieber, Frischknecht, & Waser, 1980b) assessed social interactions between two hashish-treated residents (rats housed together in two separate observation cages connected by a corridor) and an untreated intruder (a rat placed in the corridor between the two observation cages) using the same drug protocol as the previous study. In hashish-

exposed residents all categories of behaviour, except submissive behaviour and flight, appeared suppressed, although it was concluded that these behaviours might reflect the acute effects of hashish rather than anti-aggressive behaviour.

Opposite to these findings, a study by Miczek (1976) involved the chronic (5-8 weeks) administration of THC (2-50 mg/kg) in male rats. Maximum muricidal behaviour (25-70%) in previously "non-killer" rats was dependent on dose (25 mg/kg) and social manipulation (single caged).

In terms of underlying neurological mechanisms for aggressive behaviour, male CB_1 knockout mice have been found to exhibit aggression (attack frequency and duration towards an unfamiliar mouse) in a resident intruder test (Martin, Ledent, Parmentier, Maldonado, & Valverde, 2002). Further, heightened aggressive behaviour in mice devoid of the dopamine transporter (DAT) has been observed (Rodríguez de Fonseca, Chu, Caron, & Wetsel, 2004). In contrast, serotonin transporter knockout mice have shown reduced aggression in social interactions (Holmes, Murphy, & Crawley, 2003). However, the findings on the molecular bases of aggression through gene deletion appear as contradictory as the human and animal behavioural findings. This may be due to indirect effects of gene deletion, e.g. up-regulation or compensatory mechanisms may be activated in knockouts, which may alter behaviour rather than the missing gene *per se* (Nelson & Young, 1998).

Many different arguments about the possible link between cannabis and aggression have been proposed (for review see Abel, 1977): a) that cannabis is a major cause of aggression as reflected by crimes associated

with this drug, b) that an underlying predisposition towards violence might be precipitated by cannabis, c) that some psycho- or socio-pathic individuals are more likely to use various drugs, including cannabis, but there is no correlation between their behaviour and drug use, and d) that cannabis does not incite violence, but rather acts to reduce the likelihood of violent acts in those individuals under the influence of cannabis. The findings of the current study are most similar to the last proposition--cannabis use is correlated with reduced aggressive behaviour.

Although the current findings add to the controversial body of literature on the relationship between cannabis exposure and aggression, this current research differs from previous studies in that it involved the systematic analysis of aggressive behaviour across ages, and included a substantial drug-free period, ruling out drug residue in the CNS. Further, during testing, the current research was not confounded by issues such as current intoxication, stress due to chronic injections, or withdrawal effects. Lastly, this research did not involve the introduction of stressors, such as sleep deprivation, foot shock, and social manipulations. Whist the effects of cannabis on aggression in humans are not fully known, there is evidence from animal studies that cannabinoid administration can lead to a reduction of aggressive behaviour rather than an increase. At the same time, it may be the case that human cannabis use can contribute to aggressive behaviour in individuals predisposed to certain psychopathologies, although the evidence for this is still unclear at this point in time. Further, the animal literature on cannabis and aggression appears to suggest that under certain social conditions, cannabis exposure might increase irritability and aggression.

To date, animal models of aggression are mainly focused on adaptive forms of antagonistic behaviour during social interaction. Similarly, human research on aggression is largely focused on laboratory-based measures of irritability and aggressiveness. In both instances, there remains a need for more systematic comparisons of species to delineate functional and neurobiological bases of aggression (Miczek et al., 1994).

Further investigation might also examine whether synthetic cannabinioids might have some therapeutic utility over drugs such as antipsychotics and benzodiazepines, which are traditionally used to treat aggression. Indeed, some animal literature has suggested that brief periods of cannabinoid exposure during adolescence results in an increased capacity to cope with stressful situations later in life (Macri & Laviola, 2004), and decreased levels of anxiety/emotionality (Biscaia et al., 2003). These currently used drugs often have a range of side-effects and addictive potential perhaps more detrimental than the possible side-effects associated with synthetic cannabinoid drugs.

CHAPTER 7: MARGINAL ANXIOLYTIC, BUT NOT ANXIETY-LIKE BEHAVIOUR, IN ADULT RATS TREATED WITH CP 55,940 IN ADOLESCENCE

Abstract

Although anxiety is cited as a common behavioural effect of acute cannabis intoxication, few studies have systematically examined lasting anxiety 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 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 37-day drug-free period, anxiety-like behaviour (comparable to generalised anxiety in humans) was assessed using an emergence test. No significant anxiety was found, however a small, but marginally significant decrease in anxiety-like behaviour (an anxiolytic effect) was observed on only one measure (duration of time in the hide box) in adolescent CP 55,940-treated rats. This finding supports some previous cannabinoid research suggesting that cannabis use sometimes leads to a slight, but detectable, decrease in anxiety.

7.1 Introduction

One of the most commonly reported side-effects of cannabis administration in humans (Thomas, 1996) and animals is increased anxiety (Onaivi, Green, & Martin, 1990; Onaivi et al., 1995). In humans, not only has acute exposure been associated with symptoms of anxiety (Thomas, 1996), but chronic use has also been associated with a fivefold increase in the odds of reporting a state of anxiety in female users in particular (Patton et al., 2002).

In terms of underlying neural mechanisms for anxiety, it is commonly thought that the basolateral amygdala, the anterior cingulate cortex, the prefrontal cortex, and the hippocampus are implicated in anxiety-like behaviour as they are said to regulate emotional behaviour (Cahill & McGaugh, 1998). Furthermore, these regions possess high densities of CB_1 receptors (Glass, Dragunow, & Faull, 1997). In fact, recent studies (Martin et al., 2002; Urigüen, Pérez-Rial, Ledent, Palomo, & Manzanares, 2004) show that the deletion of CB_1 receptors in mice results in increased anxiety-like behaviour in the light-dark test (n.b. a variant on the emergence test, also termed "dark-light emergence test"). Further, ANA is also thought to contribute to the regulation of emotion and anxiety (Gaetani, Cuomo, & Piomelli, 2003).

To date, the acute anxiety-like effects of various cannabinoids have been well investigated (Arevalo et al., 2001; Giuliani et al., 2000; van Ree et al., 1984), as well as some lasting effects (Giuliani et al., 2000; O'Shea et al., 2004), but information on these residual effects remains sparse, and few animal models of anxiety have been used to investigate these effects.

The emergence test (see Minor et al., 1994; Morley et al., 2001; for variant see Rodríguez de Fonseca et al., 1996) measures a rat's ethological conflict between exploring a novel environment, and avoiding an open area. The apparatus consists of a smaller hide box within a larger open field. Common to exploration-based tests of anxiety is the basic premise 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 emergence test is considered to be a measure of generalised anxiety since agents such as diazepam and alprazolam alleviate anxiety in this task (Hascoët & Bourin, 1998). By the same token, it is difficult to classify animal models into separate subdivisions under the umbrella term "anxiety" as with humans (see DSM-IV-R American Psychiatric Association, 2000), especially considering that the pharmacological evidence supporting these disassociations is controversial. For example, drugs used to treat panic attack are often effective in treating GAD (Lister, 1991), and drugs used to treat depression are also effective for many types of anxiety (Handley, McBlane, Critchley, & Njung'e, 1993).

Animal models involving the measurement of unconditioned (i.e., spontaneous) behaviour, such as the emergence test, do not involve electric shock, food or water deprivation, or lengthy prior training, therefore, interference by additional variables such as intense fear, hunger/thirst, or learning/memory, are eliminated (Rodgers et al., 1997).

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).

In the current study male Wistar rats were exposed to CP 55,940 at perinatal (PND 4), adolescent (PND 30), or early adult (PND 56) ages. It was expected that the immature cannabinoid groups (i.e., the perinatal and adolescent drug-exposed groups) might show increased anxiety-like behaviour in the emergence test relative to immature controls. In adult groups, it was also anticipated that the cannabinoid group could manifest greater anxiety-like behaviour relative to adult controls.

7.2 Materials and Method

7.2.1 Subjects

As described previously (Chapter 4).

7.2.2 Drug Preparation and Administration

As described previously (Chapter 4).

7.2.3 Apparatus and Procedure

Two days after assessment in the social interaction test (Chapter 5 and 6), rats were tested in the emergence test (37 days drug-free by this stage). At the onset of testing, perinatal groups were now 62 days old, adolescent groups were 88 days old, and adult groups were 114 days old. The open field used for this experiment was a square box constructed of white melamine (120 x 120 cm x 45 cm high on three sides, and 60 cm high on the fourth side). The hide box was constructed of black melamine (40 x 25 cm x 16 cm high) with a hinged lid, and door in the front (10 cm wide x 9 cm high), which sat adjacent to the high wall of the open field with the door facing the centre of

the open field. The base of the apparatus was a vinyl floor, divided into 16 equal squares marked by black tape. A red 40 W floor lamp placed 100 cm in front of, and aimed directly at the apparatus illuminated the open field (Lux reading 010). Each 10 min trial was recorded using a black and white CCD camera with infrared illumination. At the beginning of each trial, the rat was placed in the hide box. The open field and hide box were wiped between trials with a 1:10 vinegar:water solution. An observer blind to the group allocations manually scored video recorded trials using ODLog software. Scored behaviours included emergence latency, emergence frequency, duration of time in the open field, duration of time in the hide box, number of rears, and risk assessment. Locomotor activity was measured by counting the number of transitions (line crosses) between squares in the open field of the testing apparatus.

7.3 Statistical Analysis

Unpaired *t*-tests were used to analyse each emergence test behaviour (emergence latency, emergence frequency, duration of time in the open field, duration of time in the hide box, number of rears, risk assessment, and line crosses) between the drug and vehicle groups at each age. A two-way ANOVA (age x treatment) was then used to compare behaviours across perinatal, adolescent and adult groups. Where a significant main effect of age was observed, Bonferroni-adjusted *t*-tests were used to compare age groups.

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 and ANOVAs were conducted using SPSS 12.0.1 for Windows.

7.4 Results

In perinatal rats, there were no significant differences between vehicle- and CP 55,940-treated rats in duration of emergence latency [$t(22)=1.61$, $p>0.05$], number of emergence frequencies [$t(22)<1.0$], duration of time in the open field [$t(22)<1.0$], duration of time in hide box [$t(22)<1.0$], and duration of risk assessment [$t(22)=1.10$, $p>0.05$]. It was found that locomotor activity did not differ between CP 55,940-treated rats and vehicle controls [$t(22)<1.0$] (See Figure 7.1; for statistics outputs see Appendix A35 & A38 and for data see Appendix B21 & B24).

In adolescent rats, there were no significant differences between CP 55,940-treated rats and controls in duration of emergence latency [$t(22)<1.0$], number of emergence frequencies [$t(22)<1.0$], duration of time in the open field [$t(22)=1.50$, $p>0.05$], and duration of risk assessment [$t(22)=1.36$, $p>0.05$]. Further, locomotor activity between CP 55,940-treated rats and controls did not differ [$t(22)<1.0$]. However, CP 55,940-treated rats spent less time in the hide box compared to vehicle-treated rats [$t(22)=2.23$, $p<0.05$] (See Figure 7.2; for statistics outputs see Appendix A36 & A39 and for data see Appendix B22 & B25).

In adult rats, there were no significant differences between CP 55,940-treated rats and controls in duration of emergence latency [$t(22)<1.0$], number of emergence frequencies [$t(22)=1.02$, $p>0.05$], duration of time in the open field [$t(22)<1.0$], duration of time in hide box [$t(22)=1.07$, $p>0.05$], and duration of risk assessment [$t(22)<1.0$]. Locomotor activity did not differ between CP

55,940-treated rats and controls [$t(22)=1.22$, $p>0.05$]. (See Figure 7.3; for statistics outputs see Appendix A37 & A40 and for data see Appendix B23 & B26).

Two-way ANOVAs (age x treatment) conducted on each measure yielded non-significant treatment main effects, and non-significant age by treatment interactions. There were, however, significant differences between ages in several measures. Thus, a significant main effect of age was found for emergence latency [$F(2,66)=6.06$, $p<0.01$; perinatal $M=60.49$, adolescent $M=42.15$, adult $M=56.52$], time in the open field [$F(2,66)=6.67$, $p<0.01$; perinatal $M=210.65$, adolescent $M=236.86$, adult $M=216.28$], locomotor activity [$F(2,66)=6.36$, $p<0.01$; perinatal $M=56.67$, adolescent $M=72.92$, adult $M=72.50$], and time spent in the hide box [$F(2,66)=8.77$, $p<0.001$; perinatal $M=70.05$, adolescent $M=45.55$, adult $M=61.07$]. Bonferroni-adjusted *t*-tests comparing perinatal and adolescent groups indicated that adolescent groups were slower to initially emerge [$t(46)=2.97$, $p<0.01$], spent more time in the open field [$t(46)=3.37$, $p<0.01$], displayed higher locomotor activity [$t(46)=2.85$, $p<0.01$], and spent less time in the hide box [$t(46)=3.61$, $p<0.01$]. Bonferroni-adjusted *t*-tests comparing perinatal and adult groups indicated that perinatal rats showed lower locomotor activity [$t(46)=2.99$, $p<0.01$]. Bonferroni-adjusted *t*-tests comparing adolescent and adult groups indicated that adolescent rats were significantly slower to initially emergence [$t(46)=3.23$, $p<0.01$], spent less time in the hide box [$t(46)=3.06$, $p<0.01$], and spent more time in the open field [$t(46)=2.82$, $p<0.01$].

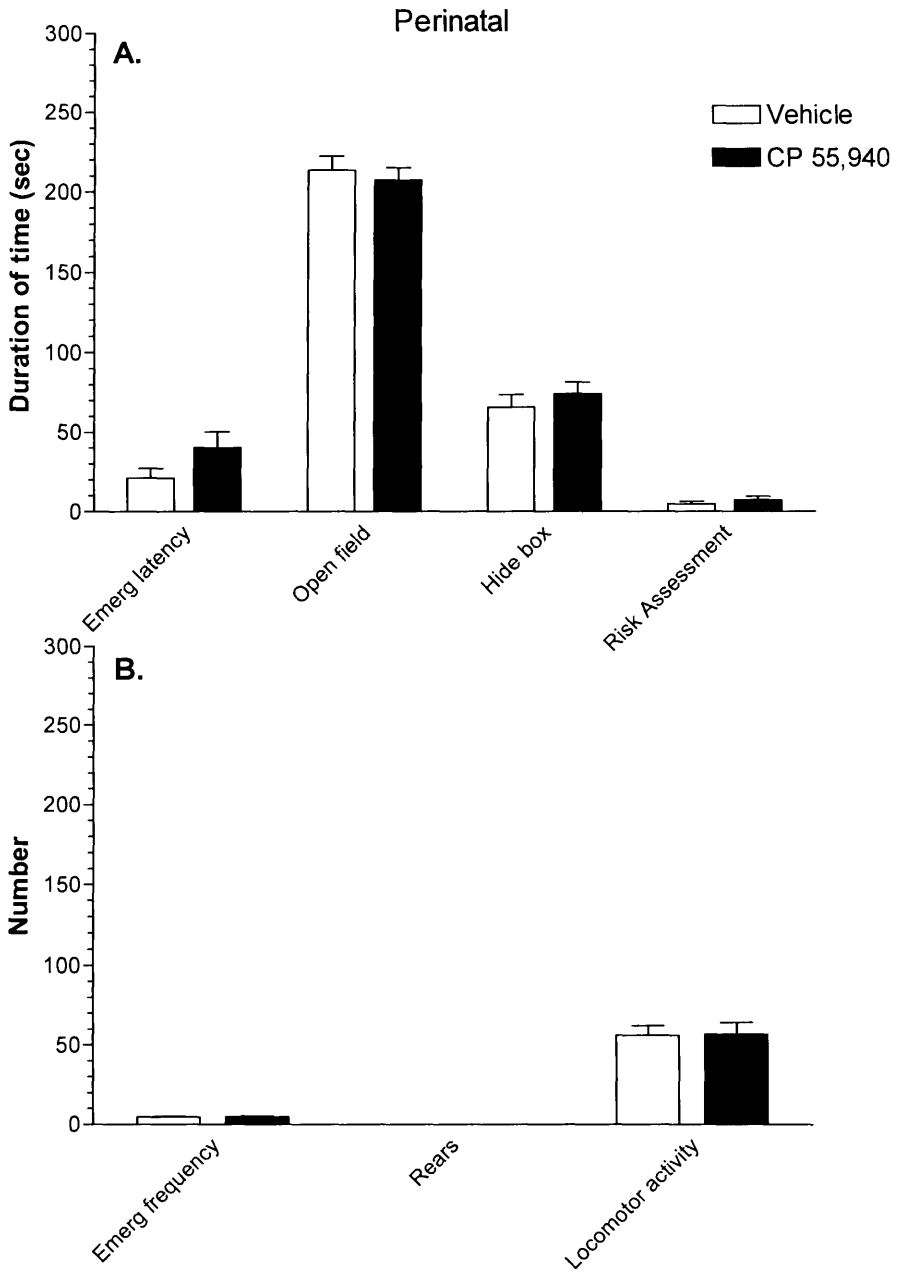

Figure 7.1 Time (sec) (A) or number of (B) emergence test behaviours in perinatal vehicle- (n=12) and CP 55,940-treated rats (n=12). Rats in half of each age group received 21 daily injections of either vehicle or CP 55,940 ending 37 days prior to testing.

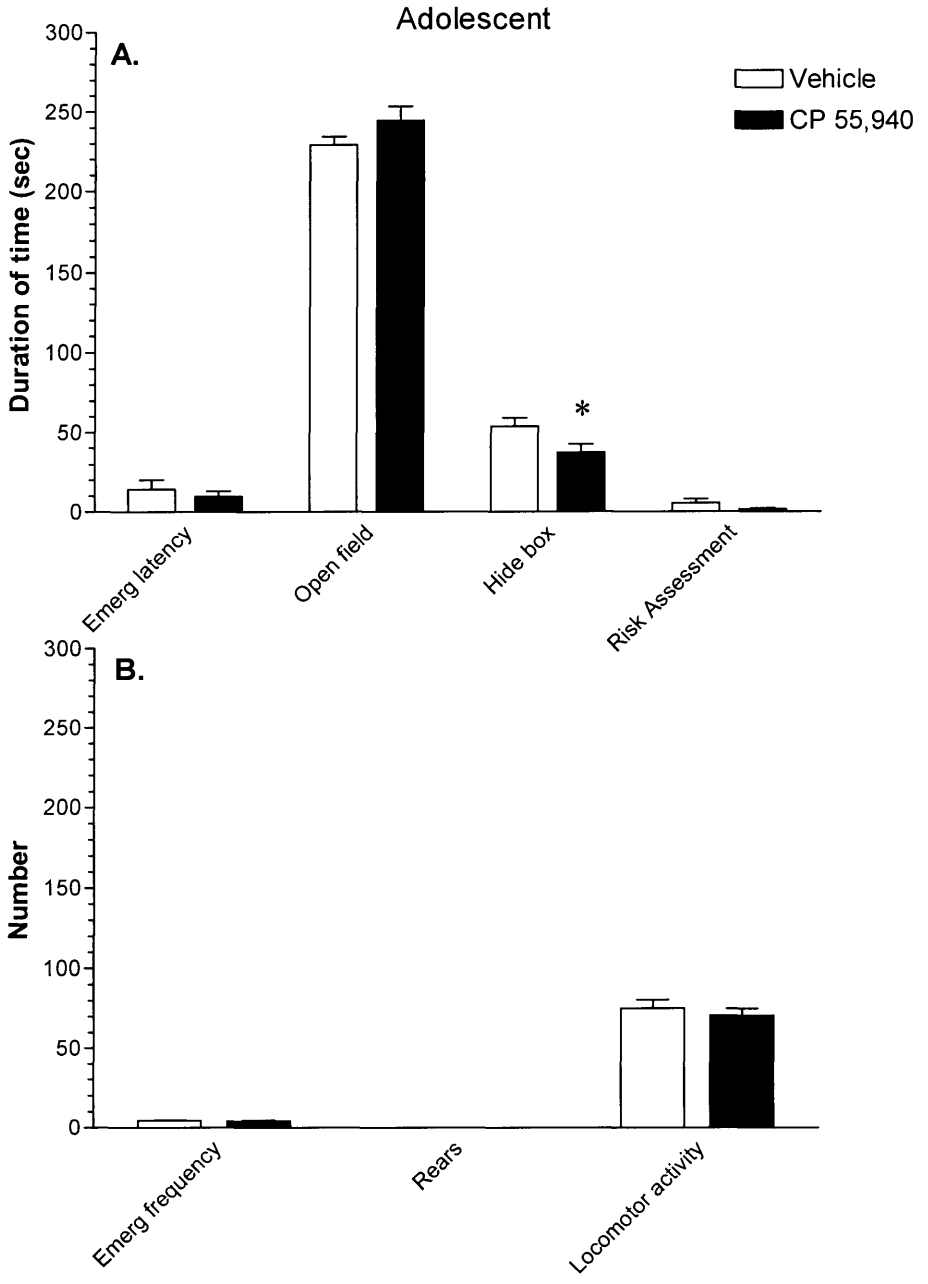

Figure 7.2 Time (sec) (A) or number of (B) emergence test behaviours in adolescent vehicle- (n=12) and CP 55,940-treated rats (n=12). Rats in half of each age group received 21 daily injections of either vehicle or CP 55,940 ending 37 days prior to testing. *CP 55,940 rats spent less time in the hide box.

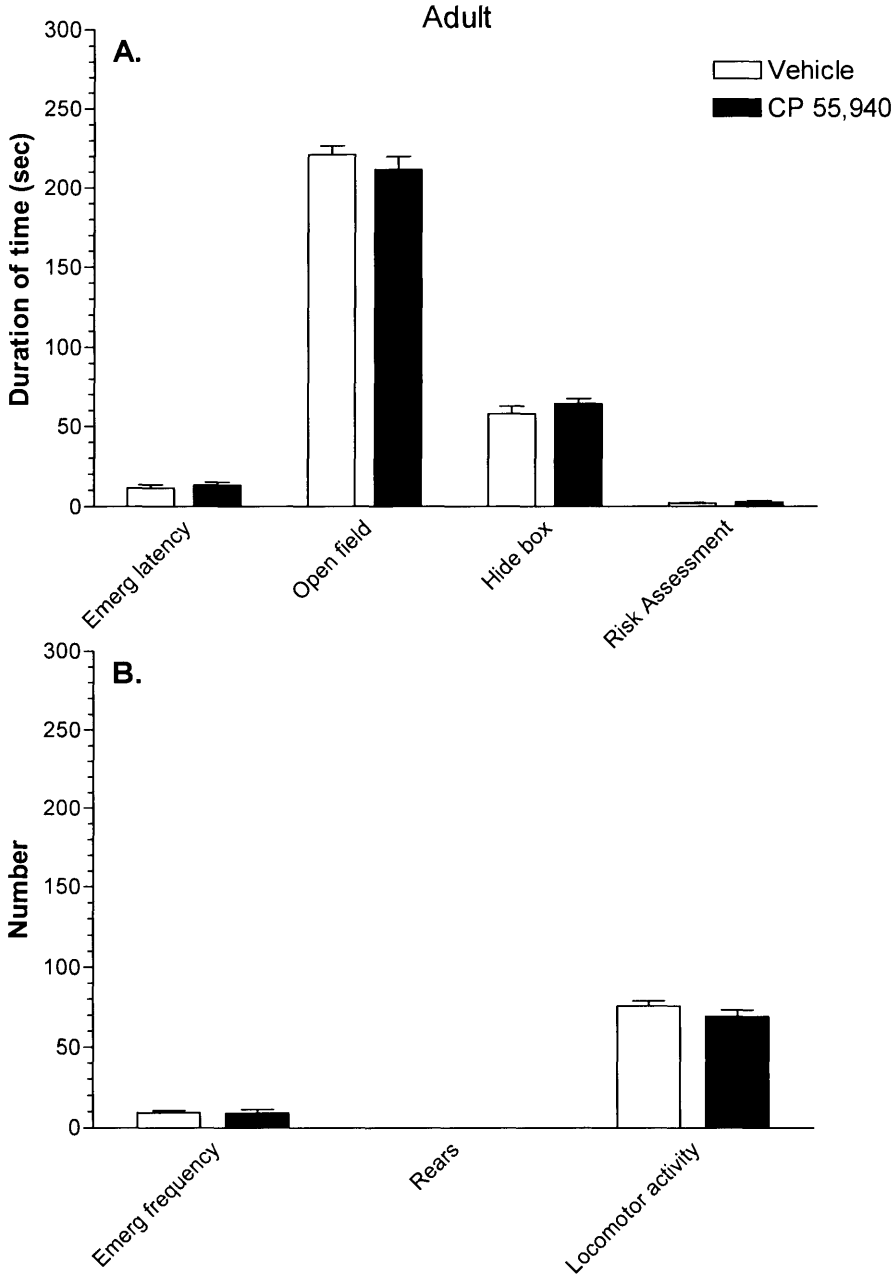

Figure 7.3 Time (sec) (A) or number of (B) emergence test behaviours in adult vehicle- (n=12) and CP 55,940-treated rats (n=12). Rats in half of each age group received 21 daily injections of either vehicle or CP 55,940 ending 37 days prior to testing.

7.5 Discussion

It was initially hypothesised that immature cannabinoid groups (i.e., the perinatal and adolescent drug-exposed groups) might show increased anxiety-like behaviour (generalised anxiety) in the emergence test relative to immature controls. In adult groups, it was also anticipated that the cannabinoid group could manifest greater anxiety-like behaviour relative to adult controls. No evidence of lasting anxiety was found, however, rats treated with CP 55,940 in adolescence showed a marginal increase in anxiolytic behaviour on one measure (duration of time in the hide box) when assessed in the emergence test in later adulthood.

The present results on perinatal cannabinoid-treated rats agree with some previous studies. One study (Navarro et al., 1994) involved THC (5 mg/kg) administration from GD 5 to PND 24 preceding assessment in the light-dark emergence test in adulthood (PND 70). Like the present study, male cannabinoid-treated rats did not exhibit increased anxiety-like behaviour in the emergence test. However, female THC-treated rats exhibited increased emergence latency indicative of anxiety-like behaviour.

A similar study involved the administration of THC (20 mg/kg) from GD 5 to PND 24, followed by behavioural testing in adulthood (PND 70) (Navarro et al., 1996). In the light-dark emergence test, performance between THC-treated rats and controls was comparable, yet THC-treated males exhibited anxiolytic (relaxed) behaviour in the social interaction test. This finding is particularly interesting, as all cannabinoid-treated groups in the current study (perinatal, adolescent, young adult) exhibited anxiety in the social interaction test three days prior to testing in the emergence test (Chapter 5). However, a

"low light, familiar test arena condition" was used, whereas Navarro et al. (1996) detected anxiolytic behaviour in the social interaction test using high light levels.

The present results showed that rats treated with CP 55,940 in adolescence exhibited decreased anxiety in later adulthood. This finding is surprising as cannabinoid exposure is commonly associated with an anxiety-provoking effect in humans (Thomas, 1996) and animals (Arevalo et al., 2001; Giuliani et al., 2000; van Ree et al., 1984). Further, acute CP 55,940 administration in male Wistar rats has been shown to lead to anxiety-like behaviour in the light-dark emergence test (Arnold, 2000).

Nonetheless, similar anxiolytic effects have been shown in adolescent CP 55,940-treated rats. For example, one study (Biscaia et al., 2003) involved the administration of CP 55,940 (0.4 mg/kg) from PND 35-45 in male and female rats. In adulthood (PND 75) these animals were tested in a holeboard test, an open field test, and an elevated plus-maze, under different stress (illumination) conditions. Sex-dependent effects were exhibited in holeboard activity, as well as a decrease in emotionality/anxiety in the open field and plus-maze.

Perhaps the current results on adolescent cannabinoid-treated rats can be partly attributed to cannabis' biphasic stimulatory effects. For example, it is sometimes found that cannabinoids can induce both anxiolytic and anxiogenic effects dependent on factors such as dose and environmental manipulation. In rats, varying acute doses of HU-210 (0.004, 0.02 and 0.10 mg/kg) have resulted in clear dose-dependent anxiety-like effects as reflected by increased emergence latency and time spent in the hide box in an emergence test (n.b.

termed "defensive withdrawal test") (Rodríguez de Fonseca et al., 1996). Another study (Genn et al., 2004) involved the administration of varying doses of CP 55,940 (0.01, 0.02, and 0.04 mg/kg) to rats, followed by testing in the social interaction test 30 min or 24 h later. This study utilised varied environmental manipulations (such as low light, familiar arena; high light, familiar arena). The results showed that the highest dose (0.04 mg/kg) produced varied anxiogenic and anxiolytic effects dependent on the environmental manipulation and time since last drug administration.

Further, although cannabis use is anxiety-provoking in humans, cannabis is also cited as contributing to a relaxant effect (Hall et al., 1994). Some animal studies support these findings. For instance, a study by Guimaraes, de Aguiar, Mechoulam, and Breuer (1994) assessed the anxiety-reducing effects of various cannabinoids such as CBD, HU-219, HU-252, HU-261, and diazepam (a pharmacological relaxant). In an elevated plus-maze, rats showed decreased anxiety similar to that produced by diazepam i.e. HU-219 (0.03-1 mg/kg) and CBD (5 mg/kg) significantly decreased anxiety, and both HU-252 and HU-261 decreased anxiety at a dose of 1 mg/kg. Similarly, another study (Onaivi et al., 1990) found that THC-treated rats exhibited aversion to open arms of an elevated plus maze (anxiety-like behaviour) whereas, CBD and nabilone administration produced an anxiolytic effect similar to that produced by diazepam.

The current results on adult rats showed that neither CP 55,940-treated rats nor controls differed on anxiety-like behaviour in the emergence test. To the author's knowledge there are no similar studies on adult rats using this paradigm, so comparisons with previous studies are not possible. However,

one previous study (Navarro et al., 1993) involved an acute dose of THC (5 mg/kg) to adult male rats (approximately 56-day old), followed by testing in the light-dark emergence test. These rats showed increased emergence latency indicative of increased anxiety-like behaviour in this test, however by the same token, THC-treated animals also took longer to escape to the hide box when placed in the open arena, perhaps reflecting a THC-induced motor deficit.

There is evidence that benzodiazepine receptors are implicated in anxiety. For example, a human study (Sethi et al., 1986) assessed 50 male chronic cannabis users (age 20-45; minimum five years use) and 50 matched controls. When evaluated on an anxiety scale, chronic cannabis users exhibited lower anxiety scores compared to controls. The authors surmised that cannabis use is commonly initiated in adolescence and early adulthood for relaxation and anti-anxiety effects. These authors then conducted an animal study to determine whether there is an association between the anti-anxiety effects of cannabis and benzodiazepine receptors. Mice received acute or chronic (100 mg/kg) cannabis treatment followed by behavioural testing. Chronic cannabis treatment (28 days) resulted in foot shock induced aggression, which was subsequently reduced by a benzodiazepine receptor antagonist. Specific 3H-diazepam binding was then carried out in the frontal cortex to assess both the population and affinity of benzodiazepine receptors. Acute cannabis treatment had no significant effect, whereas chronic cannabis treatment significantly increased 3H-diazepam binding. The authors concluded that the anti-anxiety effects of cannabis are mediated through central benzodiazepine receptors.

Overall, the present findings should be interpreted with caution as they might simply reflect a floor effect i.e. rats emerged after a very brief latency, and spent increased time in the open field, perhaps failing to discriminate anything anxiogenic in the apparatus. Conflicting results between the social interaction and emergence tests, however, are not uncommon (Navarro et al., 1994; Navarro et al., 1996). This disparity has been attributed to distinct facets of anxiety (i.e., social anxiety in the social interaction test and generalised anxiety in the emergence test) that may be differentially susceptible to drug treatments (Clemens et al., 2004). Results from experiments using animal tests of anxiety such as the emergence and light-dark tests are also frequently age- and sex-dependent. For example, a study comparing male mice of various ages found lower anxiety only in the youngest and oldest mice (Hascoët et al., 1999). The authors suggested that the differences might be related to deficits in brain maturity for younger animals, and functional deficits in older animals. Sex-specific effects have been noted in other animal paradigms (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 test used (Biscaia et al., 2003). Further, various versions of both the light-dark and emergence tests makes it difficult to compare results across paradigms. Thus, caution needs to be exercised in the interpretation of results as these may be sex-, age-, or paradigm-specific. More investigation of cannabis' anxiolytic effects also warrants further enquiry, as cannabinoids such as CP 55,940, are traditionally classed as anxiogenic rather than anxiolytic agents.