CHAPTER 8: THE ASSESSMENT OF CANNABINOID-INDUCED NEURONAL CHANGE USING C-FOS IMMUNOHISTOCHEMISTRY

Abstract

Previous work using *c-fos* immunohistochemistry identified lasting alterations of basal neuronal activity following perinatal (Singh et al., In press) and adult (Singh et al., 2005) onset cannabinoid exposure. However, the methods used in these previous studies could not rule out the possibility that altered basal *c-fos* expression may simply have reflected a change in brain activity due to a conditioned drug state. The aim of the present study was to further examine basal *c-fos* expression in cannabinoid-exposed rats across a wider age range using confound-free methods. 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 39-day drug-free period, *c-fos*-immunoreactive cells were quantified in several brain regions. Interestingly, differing baseline levels of *c-fos* expression dependent on age were observed in the piriform cortex, NAC shell, ventral LS, central and medial nucleus of the amygdala, dorsolateral and lateral PAG, and the CA3 region of the hippocampus. No long-term drug-induced alterations in basal *c-fos*-immunoreactivity were found between cannabinoid and vehicle groups at any ages. These results suggest that previous evidence of long-term cannabinoid-induced alterations of neuronal activity using *c-fos* immunohistochemistry may have been conditioned drug effects.

8.1 Introduction

Cannabis is the most widely produced, trafficked, and consumed illicit drug worldwide (United Nations Office on Drugs and Crime, 2003) and one of the most popularly used illicit drugs during pregnancy (Fried & Smith, 2001). In both humans and animals, the acute and chronic cognitive effects of cannabis administration have been well researched (for review see Solowij, 1998). 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 when onset occurs at major developmental ages. So far, behavioural studies on the lasting effects of cannabis on animals (Gianutsos & Abbatiello, 1972; Kawash et al., 1980; Mereu et al., 2003) and humans (Fried et al., 2003; Richardson et al., 2002) suggest that perinatal cannabinoid exposure is associated with cognitive deficits. Likewise, the animal literature suggests that adolescent (Fehr et al., 1976; Fehr et al., 1978; Stiglick & Kalant, 1982a; Stiglick & Kalant, 1982b; Stiglick & Kalant, 1983), but not adult (Stiglick & Kalant, 1985) onset cannabinoid exposure is associated with cognitive deficits in later life. Further, recent animal studies involving direct adolescent-adult comparisons have shown cognitive deficits specific to adolescent onset exposure (O'Shea et al., 2004; Schneider & Koch, 2003). In humans, early but not late adolescent onset exposure has been associated with lasting cognitive deficits (Ehrenreich et al., 1999; Pope et al., 2003).

Neurological investigation of cannabis' lasting effects has largely focused on perinatal cannabinoid exposure in rats. To date, this literature suggests that cannabinoid exposure is indeed associated with neurological abnormalities (Fernández-Ruiz et al., 1992; Fernández-Ruiz et al., 1997;

Fernández-Ruiz et al., 1999; Fernández-Ruiz et al., 2000). These investigations have largely focused on dopamine receptor change (i.e., alterations in dopamine neurotransmission) and cannabinoid receptor development, where currently there is mounting evidence that the most pronounced neurobehavioural change is demonstrated in males (for review see Fernández-Ruiz et al., 1992; Moreno et al., 2003; Navarro et al., 1996). More recently cannabinoid exposure in male, but not female rats has been associated with changes in the neural adhesion molecule L1 (Gomez et al., 2003).

Other studies have found mixed evidence of neurological change in animals of a slightly older age. Chronic cannabis administration in adult rats and adolescent monkeys failed to alter cannabinoid receptor populations in the brains of both species following a substantial drug-free period (Westlake et al., 1991). Another study on adult rats (Scallet et al., 1987) used light and electron microscopy to quantify the effects of chronic THC on the anatomical structure of the hippocampus. Following a 7-month drug-free period, the ultrastructural appearance of neurons was altered and the volume of neurons and their nuclei in the CA3 region were reduced. Further, a 44% reduction in the number of synapses per unit volume was found. Golgi impregnation studies of additional adult rats treated with THC revealed a reduction in dendritic length of CA3 pyramidal neurons, despite no apparent changes in ultrastructure and no changes in synaptic density.

These same investigators (Ali et al., 1989), examined the effects of chronic THC exposure on various neurotransmitter systems (i.e., dopamine, serotonin, acetylcholine, GABAergic, benzodiazepine, and opiate). Two

months after the cessation of chronic THC administration, a significant decrease in GABA receptor binding in the hippocampus of animals was observed, however, this GABA receptor binding was not replicated in another experiment. Another study (Landfield et al., 1988) found an association between chronic THC-exposure and hippocampal change (an increase of cytoplasmic inclusions).

Altered brain function and metabolism in humans following acute and chronic cannabis exposure has been observed using imaging techniques such as CBF, PET, and ERP. For example, a case study by Solowij et al. (1995) examined whether cessation of cannabis use leads to recovery of known attention deficits. Selective attention in an individual with an 18-year history of cannabis use was assessed at 1, 3, and 6 weeks post-cessation as measured by brain ERP. It was found that there was no recovery of selective attention following 6 weeks cessation. Generally, the results of human studies suggest subtle cannabis-induced brain dysfunction; however, existing imaging methods are not sensitive enough to detect subcellular alterations (for review see Solowij, 1998).

Although the findings on cannabis effects on brain morphology are mixed, there is some evidence that cannabinoids could cause lasting detrimental effects on the brain. For instance, a study by Chan, Hinds, Impey, and Storm (1998) suggested that THC is toxic to hippocampal neurons. This particular study involved treatment of cultured neurons or hippocampal slices with THC. Treatment with THC was found to cause shrinkage of neuronal cell bodies and nuclei as well as genomic deoxyribonucleic acid (DNA) strand breaks, characteristic of neuronal apoptosis. Interestingly, neuronal death

induced by THC was inhibited by nonsteroidal anti-inflammatory drugs, including aspirin and indomethacin, as well as vitamin E and other antioxidants.

Previous work conducted in the author's laboratory found altered Fos-IR in several brain regions of rats exposed to THC during perinatal (Singh et al., In press) and early adult (Singh et al., 2005) developmental periods. These results were thought to provide evidence that brain function was altered in a relatively permanent manner by cannabinoid exposure. However, the methods used in this previous work prevented any clear conclusions to be drawn. That is, the observed drug-induced alterations in Fos-IR may simply have been the product of a conditioned drug effect (Singh et al., In press, 2005). The present study attempts to settle this matter by examining basal *c-fos* immunohistochemistry in several brain regions of rats exposed to CP 55,940 or its vehicle during perinatal, adolescent and adult ages.

In the current study male Wistar rats were exposed to CP 55,940 for 21 days starting on PND 4, PND 30, or PND 56. Following a 39-day drug-free period, *c-fos* immunohistochemistry was used to detect differences in neuronal activation between CP 55,940- and vehicle-treated groups at each developmental age.

The immunohistochemical labelling of *c-Fos* is considered to be a reliable metabolic marker of neuronal activation (Dragunow & Faull, 1989). A wide range of drugs such as cocaine, morphine, hallucinogens, amphetamines, ethanol, caffeine, nicotine, and THC have been found to produce clear patterns of *c-fos* expression in distinct areas of the brain (for review see Harlan & Garcia, 1998). Over the last decade a number of

immediate early genes and associated proteins have been discovered in the brain that are believed to play a role in neuronal plasticity (for review see Hughes & Dragunow, 1995). *C-fos* immunohistochemistry is facilitated through an antigen-antibody reaction on brain tissue. This reaction can be visualised by enzymatic colour reactions, and allows the examination of neuronal change as facilitated through the staining of affected neurons in brain regions and subregions of interest.

To this end, *c-fos*-immunoreactive cells were counted in specific brain regions and subregions. It was predicted that the immature CP 55,940-treated groups (perinatal, adolescent) would exhibit altered *c-fos*-immunoreactivity relative to immature controls due to altered neuronal functioning produced by cannabinoids during early development. In the adult rats, it was anticipated that significant differences in *c-fos*-immunoreactivity might also be exhibited between the CP 55,940- and vehicle-treated groups irrespective of mature age at exposure. An increase in Fos-IR-labelled cells in cannabinoid-treated rats is expected to illustrate lasting changes in the CNS due to previous cannabinoid exposure. Previously these same rats exhibited behavioural changes in paradigms such as the object recognition task (Chapter 4) the social interaction test (Chapter 5 and 6), and the emergence test (Chapter 7). In the object recognition task, working memory was impaired in perinatal, adolescent, and adult CP 55,940-treated rats. In the social interaction test, perinatal, adolescent, and adult CP 55,940-treated rats showed reduced social behaviours; and adolescent and adult CP 55,940-treated rats showed reduced aggressive behaviours compared with controls.

In the emergence test, rats treated with CP 55,940 in adolescence showed marginal evidence of lasting anxiolytic behaviour (decreased anxiety).

8.2 Materials and Method

8.2.1 Subjects

Of the original 72 male Wistar rats in the previous behavioural studies, 48 of these were used. There were 16 rats in the perinatal groups, 16 rats in the adolescent groups, and 16 rats in the adult groups. At each developmental age, 8 of these rats comprised the drug-exposed group and the other 8 served as vehicle controls.

8.2.2 Drug Preparation and Administration

As specified in Chapter 4.

8.2.3 Apparatus and Procedure

Two days after assessment in the emergence test (Chapter 7) ended (39 days drug-free by this time), perinatal groups were now 64 days old, adolescent groups were 90 days old, and adult groups were 116 days old. Rats were injected with sodium pentobarbitone (120 mg/kg), and were perfused transcardially with 120 ml of 0.1 M phosphate-buffed saline (PBS, pH 7.2), followed by 150 ml of 4 % paraformaldehyde in PBS. Brains were then extracted and stored overnight at 4°C in paraformaldehyde solution. The following day brains were placed in 15% sucrose at 4°C for 24 h, then in 30% sucrose at 4°C for 48 h. Whole brains were then sectioned at 40 µm using a cryostat, and placed in phosphate buffer (PB, pH 7.2). Free-floating sections were incubated for 30 min in a hydrogen peroxide (0.9 %) solution, then in normal horse serum (3%) for a further 30 min. Sections were then incubated with the primary *c-fos* antibody (rabbit polyclonal; Santa Cruz Biotechnology)

diluted 1:2000 in phosphate buffed horse serum (0.1% bovine serum albumin, 0.2% Triton X-100, 2% normal horse serum in PB) at 4°C for 72 h, rinsed in PB for 30 min, and placed in biotinylated anti-rabbit IgG (Vector Laboratories) for 1 h at a dilution of 1:500 in phosphate-buffered horse serum. Brain slices were washed in PB for another 30 min then incubated for 2 h in ExtrAvidin-horseradish peroxidase (Sigma; diluted at 1:1000 in phosphate buffed horse serum). After three 30-min washes in PB, horseradish peroxidase activity was visualised using the nickel diaminobenzidine and glucose oxidase reaction, which was terminated after 10 min by rinsing with PB. Next, sections were mounted onto subbed slides, dehydrated, Histolene cleared, and cover-slipped. Sections were then examined using an Olympus CH-2 microscope set at 200 X magnification to quantify the number of labelled nuclei. A total of 15 brain regions or subregions were examined (See Figure 8.1- 8.6). Brain regions were selected either because they have been implicated in anxiety or learning and memory, or because previous work revealed that *c-fos* expression was influenced in that region by acute cannabinoid administration (Allen, McGregor, Hunt, Singh, & Mallet, 2003; Arnold, Topple, Mallet, Hunt, & McGregor, 2001).

Brain regions were identified using the atlas of Paxinos and Watson (1998). Where structures were not readily visible the location was determined by examining the distance relative to surrounding visible structures. A 0.5 mm square graticule was positioned over each structure and the number of dark-brown to black Fos-labelled nuclei was counted manually. Where the structure exceeded the size of the graticule a standardised region within this

structure was counted. Slides were coded such that the person quantifying labelled nuclei was unaware of treatment allocations.

Fos-labelled cells were photographed using a cooled Nikon Digital Sight imaging system attached to a Nikon Eclipse 55i microscope. Post-production of images involved the standardisation of picture quality for printing purposes using Deneba Canvas 9.0

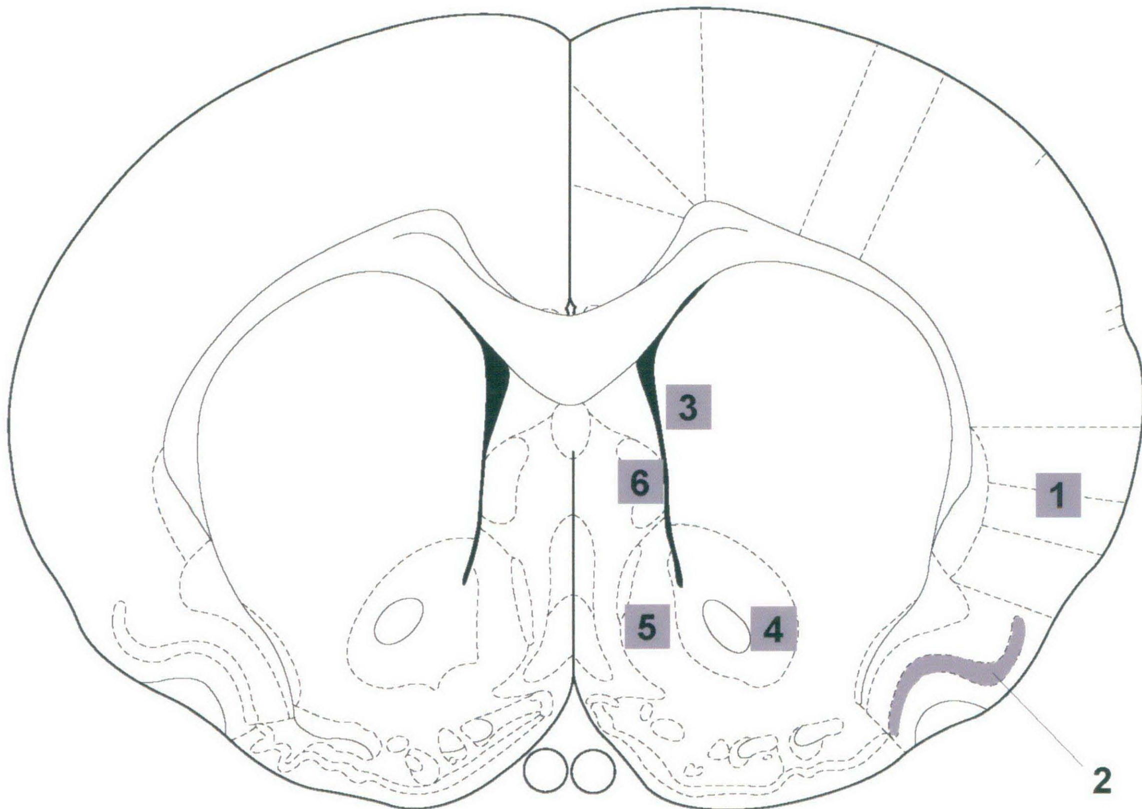

Figure 8.1 Schematic diagram of a coronal section of rat brain at Bregma +1.00 (Paxinos and Watson, 1998). The number of Fos-labelled nuclei were counted within the areas numbered and shaded in gray representing the graticule placement. Areas were as follows: 1) insular cortex, 2) piriform cortex, 3) medial CPU, 4) NAC core, 5) NAC shell, and 6) ventral LS.

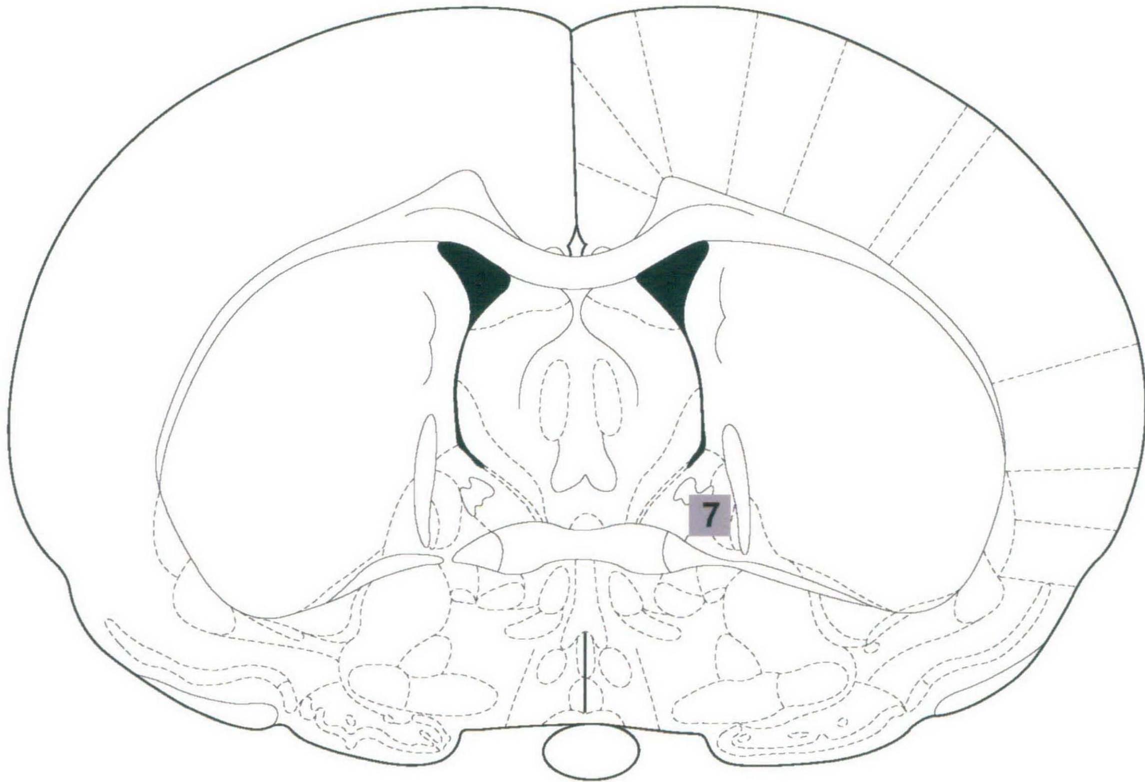

Figure 8.2 Schematic diagram of a coronal section of rat brain at Bregma -0.26 (Paxinos and Watson, 1998). The number of Fos-labelled nuclei were counted within the areas numbered and shaded in gray representing the graticule placement. The area examined at this location was: 7) dorsal BNST lateral division.

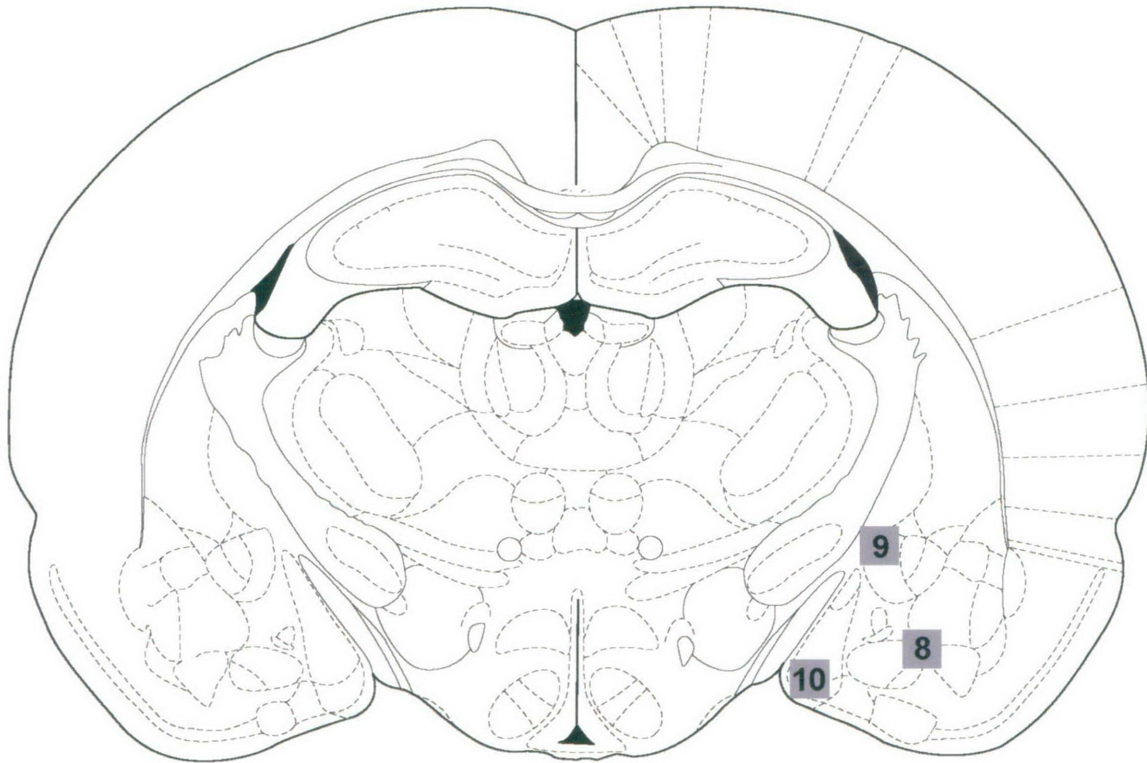

Figure 8.3 Schematic diagram of a coronal section of rat brain at Bregma -2.80 (Paxinos and Watson, 1998). The number of Fos-labelled nuclei were counted within the areas numbered and shaded in gray representing the graticule placement. Areas were as follows: 8) basolateral nucleus of the amygdala, 9) central nucleus of the amygdala, and 10) medial nucleus of the amygdala.

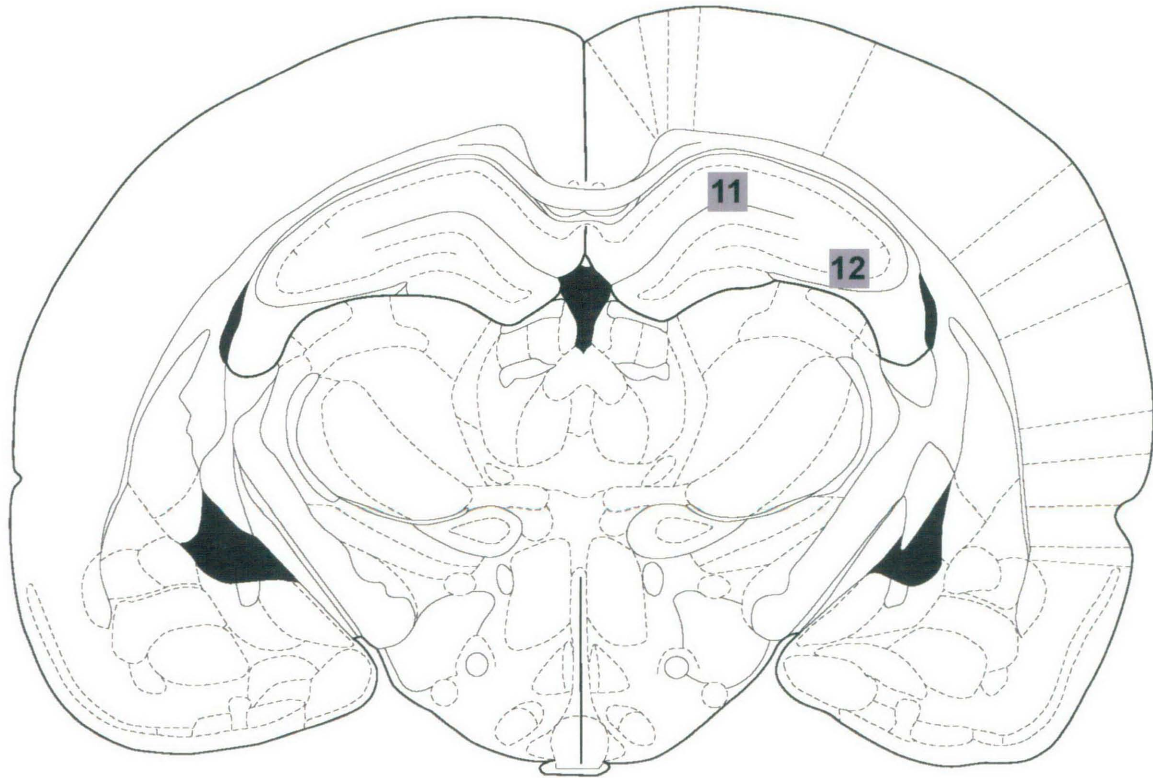

Figure 8.4 Schematic diagram of a coronal section of rat brain at Bregma -3.60 (Paxinos and Watson, 1998). The number of Fos-labelled nuclei were counted within the areas numbered and shaded in gray representing the graticule placement. Areas examined were: 11) hippocampus CA1 and 12) hippocampus CA3.

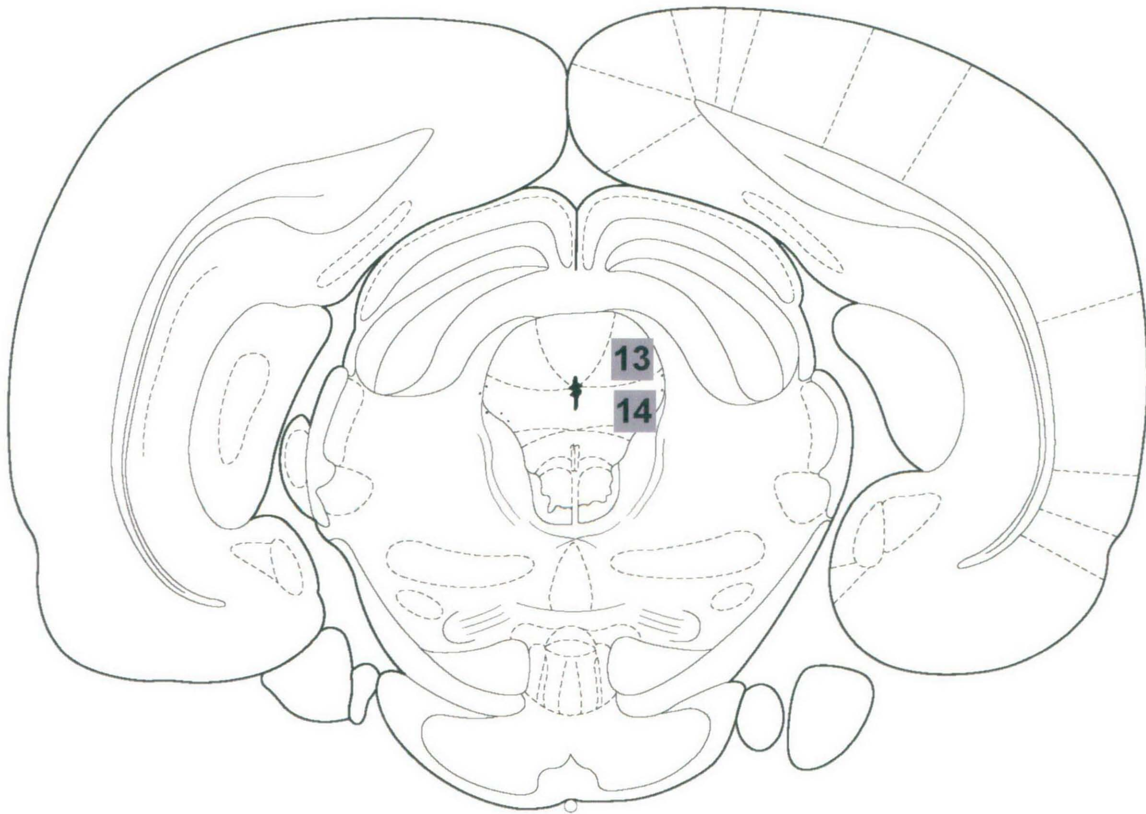

Figure 8.5 Schematic diagram of a coronal section of rat brain at Bregma -6.72 (Paxinos and Watson, 1998). The number of Fos-labelled nuclei were counted within the areas numbered and shaded in gray representing the graticule placement. Areas were as follows: 13) PAG dorsolateral and 14) PAG lateral.

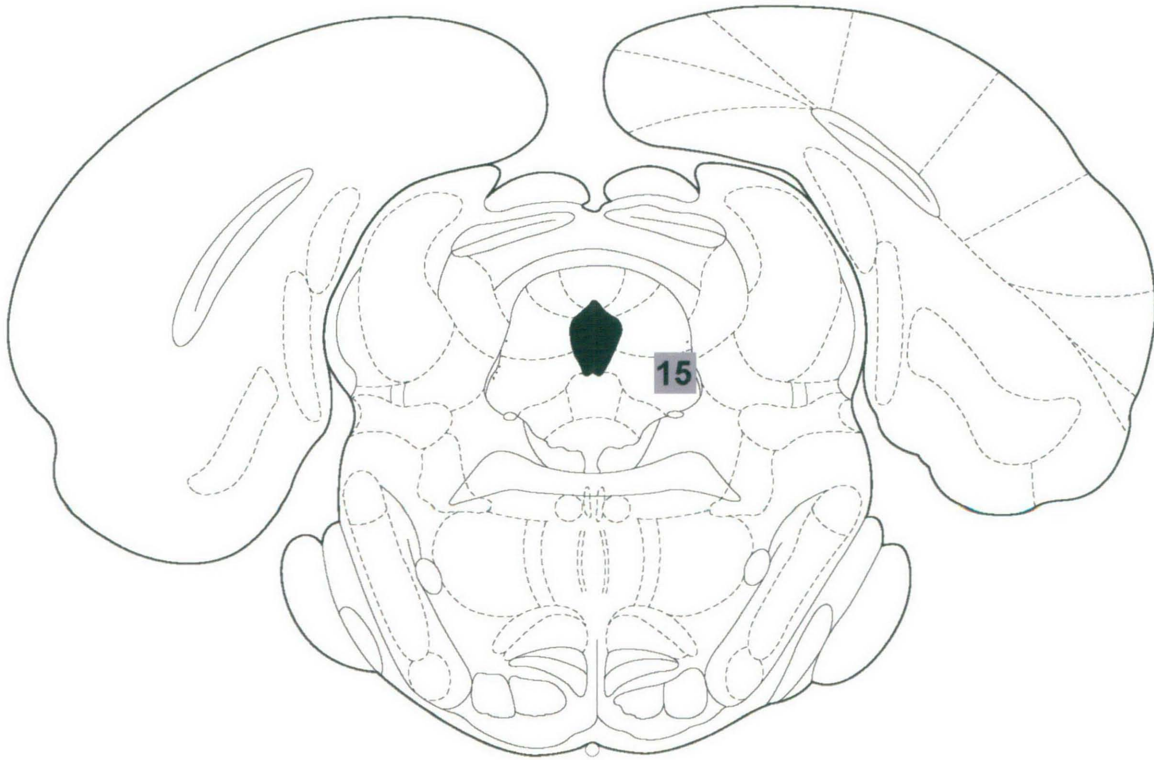

Figure 8.6 Schematic diagram of a coronal section of rat brain at Bregma -8.00 (Paxinos and Watson, 1998). The number of Fos-labelled nuclei were counted within the areas numbered and shaded in gray representing the graticule placement. The area examined at this location was: 15) PAG ventrolateral.

8.3 Statistical Analysis

Separate *t*-tests were used to compare the number of labelled nuclei between treatments within each age group. A two-way ANOVA (age x treatment) was used to compare immunoreactive cells between perinatal, adolescent and adult groups. Where main effects of age were observed, *post hoc* Tukey tests were conducted. 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.

8.4 Results

The number of Fos-immunoreactive cells for the 15 brain regions and subregions examined are presented in Table 8.1. *t*-tests comparing perinatal CP 55,940-treated rats and vehicle controls indicated that there were no significant differences in Fos-labelled cells in the insular cortex [$t(14)<1.0$], piriform cortex [$t(14)<1.0$], medial CPU [$t(14]=1.85$, $p>0.05$], core of the NAC [$t(14]=1.32$, $p>0.05$], shell of the NAC [$t(14)<1.0$], ventral part of the LS [$t(14]=1.09$, $p>0.05$], dorsal BNST lateral division [$t(14)<1.0$], basolateral nucleus of the amygdala [$t(14)<1.0$], central nucleus of the amygdala [$t(14]=1.59$, $p>0.05$], medial nucleus of the amygdala [$t(14)<1.0$], hippocampus CA1 [$t(14)=1.43$, $p>0.05$], hippocampus CA3 [$t(14)<1.0$], dorsolateral PAG [$t(14]=1.72$, $p>0.05$], lateral PAG [$t(14)<1.0$], and ventrolateral PAG [$t(14)<1.0$] (For statistics outputs see Appendix A41 and for data see Appendix B27).

Table 8.1 Mean number (± SEM) of Fos-labelled cells in each structure by drug treatment (n = 8 per vehicle and CP 55,940 treatment group) for perinatal, adolescent, and adult rats.

Region	Bregma	Perinatal		Adolescent		Adult		Stats
		Vehicle	CP 55,940	Vehicle	CP 55,940	Vehicle	CP 55,940	
1. insular cortex	+1.00	1.0 ± 0.5	1.1 ± 0.5	0.3 ± 0.2	0.4 ± 0.3	2.3 ± 0.1	1.3 ± 0.8	
2. piriform cortex	+1.00	30.4 ± 7.6	37.0 ± 9.2	9.4 ± 3.3	12.0 ± 8.4	23.1 ± 8.7	19.4 ± 4.5	*, $p<0.05$
3. medial CPU	+1.00	1.1 ± 0.5	3.9 ± 1.4	0.4 ± 0.3	0.5 ± 0.3	1.3 ± 0.8	1.6 ± 1.2	
4. NAC core	+1.00	0.0 ± 0.0	1.0 ± 0.8	0.4 ± 0.2	0.4 ± 0.3	0.5 ± 0.4	1.0 ± 0.7	
5. NAC shell	+1.00	0.4 ± 0.3	1.0 ± 0.6	0.1 ± 0.1	0.3 ± 0.2	3.8 ± 1.8	2.1 ± 1.1	*, $p<0.01$
6. LS, ventral	+1.00	5.5 ± 2.1	9.8 ± 3.3	4.0 ± 1.2	3.0 ± 0.8	2.0 ± 1.2	2.1 ± 0.7	*, $p<0.01$
7. BNST lateral division, dorsal	-0.26	0.4 ± 0.4	0.1 ± 0.1	1.4 ± 0.8	1.1 ± 0.9	0.6 ± 0.5	0.0 ± 0.0	
8. basolateral nucleus of amygdala	-2.80	3.5 ± 0.8	2.5 ± 1.1	1.4± 0.7	1.3 ± 0.7	2.9 ± 1.9	1.0 ± 0.6	
9. central nucleus of amygdala	-2.80	6.8 ± 2.0	3.1 ± 1.1	1.0 ± 0.6	1.3 ± 0.6	2.0 ± 0.8	3.3 ± 0.9	*, $p<0.01$
10. medial nucleus of amygdala	-2.80	7.0 ± 2.5	8.6 ± 1.9	4.0 ± 1.5	3.5 ± 2.0	2.9 ± 1.2	5.1 ± 1.1	*, $p<0.05$
11. hippocampus CA1	-3.60	0.0 ± 0.0	0.4 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.3	0.3 ± 0.2	
12. hippocampus CA3	-3.60	0.5 ± 0.3	0.5 ± 0.3	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	*, $p<0.05$
13. PAG dorsolateral	-6.72	7.0 ± 1.7	3.4 ± 1.2	1.6 ± 0.8	1.8 ± 0.5	2.4 ± 1.2	1.1 ± 0.2	*, $p<0.01$
14. PAG lateral	-6.72	7.4 ± 2.2	4.9 ± 1.7	0.4 ± 0.4	0.4 ± 0.2	0.5 ± 0.3	0.6 ± 0.3	*, $p<0.001$
15. PAG ventrolateral	-8.00	6.4 ± 2.5	9.5 ± 2.9	4.0 ± 1.4	5.9 ± 2.0	3.6 ± 1.7	2.8 ± 1.1	

*Significant main effect of age.

In adolescent CP 55,940 and vehicle treatment groups there were no significant differences in the insular cortex [$t(14)<1.0$], piriform cortex [$t(14)<1.0$], medial CPU [$t(14)<1.0$], core of the NAC [$t(14)<1.0$], shell of the NAC [$t(14)<1.0$], ventral part of the LS [$t(14)<1.0$], dorsal BNST lateral division [$t(14)<1.0$], basolateral nucleus of the amygdala [$t(14)<1.0$], central nucleus of the amygdala [$t(14)<1.0$], medial nucleus of the amygdala [$t(14)<1.0$], hippocampus CA1 [no *t*-tests result as means=0], hippocampus CA3 [$t(14)=1.00$, $p>0.05$], dorsolateral PAG [$t(14)<1.0$], lateral PAG [$t(14)<1.0$], and ventrolateral PAG [$t(14)<1.0$] (For statistics outputs see Appendix A42 and for data see Appendix B28).

In adult rats there were no significant differences between CP 55,940 and vehicle groups in the insular cortex [$t(14)<1.0$], piriform cortex [$t(14)<1.0$], medial CPU [$t(14)<1.0$], core of the NAC [$t(14)<1.0$], shell of the NAC [$t(14)<1.0$], ventral part of the LS [$t(14)<1.0$], dorsal BNST lateral division [$t(14)=1.26$, $p>0.05$], basolateral nucleus of the amygdala [$t(14)<1.0$], central nucleus of the amygdala [$t(14)=1.00$, $p>0.05$], medial nucleus of the amygdala [$t(14)=1.42$, $p>0.05$], hippocampus CA1 [$t(14)<1.0$], hippocampus CA3 [$t(14)=1.00$, $p>0.05$], dorsolateral PAG [$t(14)<1.0$], lateral PAG [$t(14)<1.0$], and ventrolateral PAG [$t(14)<1.0$] (For statistics outputs see Appendix A43 and for data see Appendix B29).

Separate two-way (age x treatment) ANOVAs conducted for each brain region revealed no significant treatment main effects, and no significant age by treatment interactions across all brain regions. However, significant main effects of age were observed for the piriform cortex [$F(2,42)=4.99$, $p<0.05$], NAC shell [$F(2,42)=5.45$, $p<0.01$], ventral LS [$F(2,42)=5.22$, $p<0.01$], central

and [$F(2,42)=5.94$, $p<0.01$] medial nucleus of the amygdala [$F(2,42)=3.31$, $p<0.05$], dorsolateral [$F(2,42)=7.02$, $p<0.01$] and lateral divisions [$F(2,42)=15.31$, $p<0.001$] of the PAG, and the CA3 region of the hippocampus [$F(2,42)=4.40$, $p<0.05$] (see Table 8.2).

Table 8.2 Mean number (SEM) of Fos-IR-labelled cells in select brain regions of perinatal, adolescent, and adult rats. The means are averaged across vehicle and CP 55,940 treatment groups at each age.

Age	Piriform cortex	NAC shell	LS ventral	Amygdala, central nucleus	Amygdala, medial nucleus	PAG, dorsolateral	PAG, lateral	Hippocampus CA3
Perinatal	33.7±5.8	0.7±0.3	7.6±2.0	4.9±1.2	7.8±1.5	5.2±1.1	6.1±1.4	0.5±0.2
Adolescent	10.7±4.4	0.2±0.1	3.5±0.7	1.1±0.4	3.8±1.2	1.7±0.4	0.4±0.2	0.1±0.1
Adult	21.3±4.7	2.9±1.0	2.1±0.7	2.6±0.6	4.0±0.8	1.8±0.6	0.6±0.2	0.1±0.1

Post hoc Tukey tests comparing ages revealed that *c-fos*-immunoreactivity was significantly lower in the adolescent rats relative to the perinatal rats for the piriform cortex. *C-fos*-immunoreactivity was higher in adult rats relative to both perinatal and adolescent rats for the NAC shell, while *c-fos*-immunoreactivity was significantly higher in the perinatal rats relative to the adult rats for the ventral LS. The number of immunoreactive cells was significantly higher in perinatal rats relative to adolescent rats in the central nucleus of the amygdala. No *post hoc* age effects were significant for the medial nucleus of the amygdala. Finally, *c-fos*-immunoreactivity was significantly higher in the perinatal rats relative to both the adolescent and adult rats for the PAG (dorsolateral and lateral divisions), and the CA3 region of the hippocampus (See Figures 8.7- 8.13 for photomicrographs of these regions and subregions).

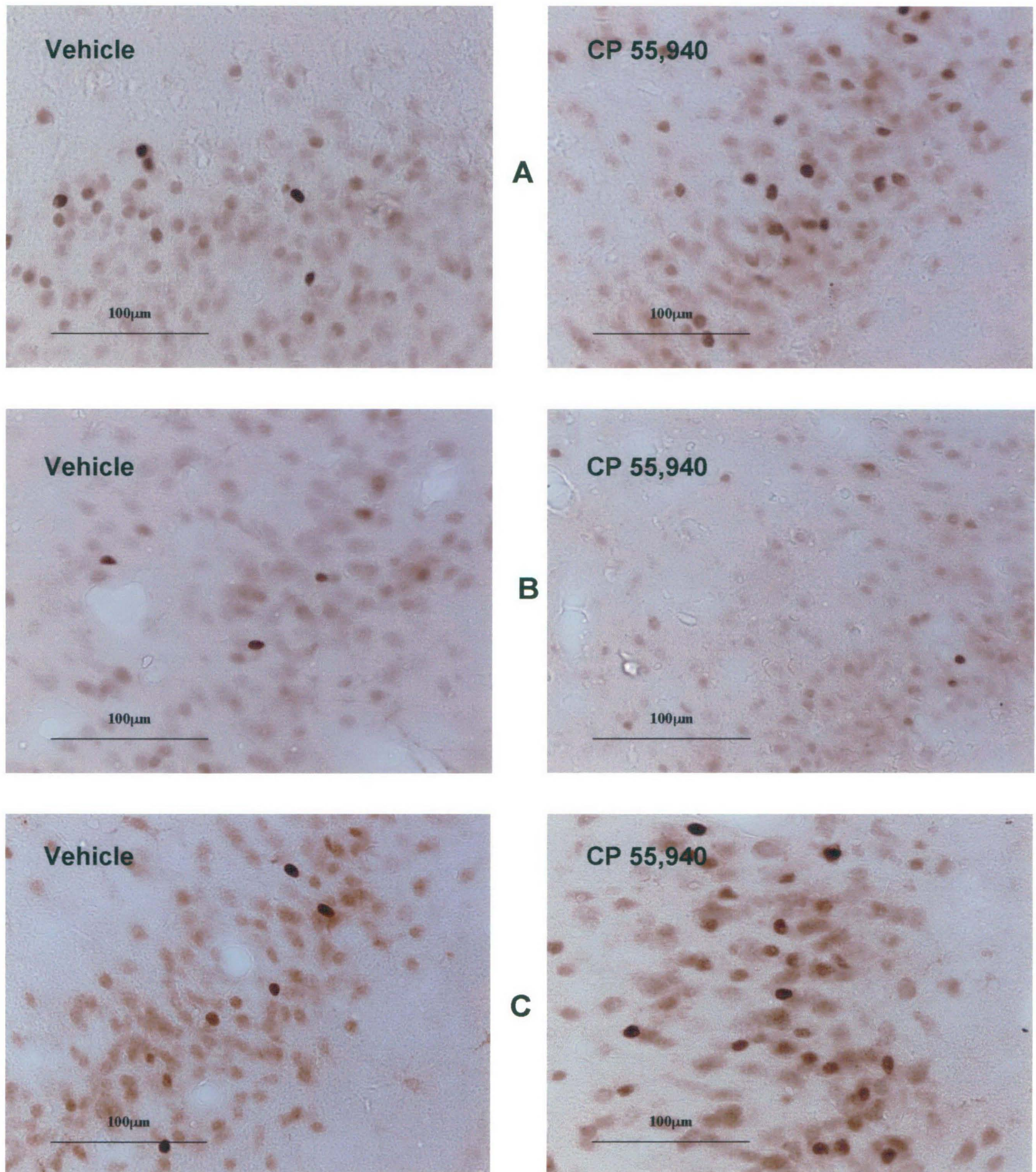

Figure 8.7 Photomicrograph of immunoreactive cells in the piriform cortex for A) perinatal, B) adolescent, and C) adult groups. Higher Fos immunoreactivity was observed in perinatal groups compared to adolescent groups.

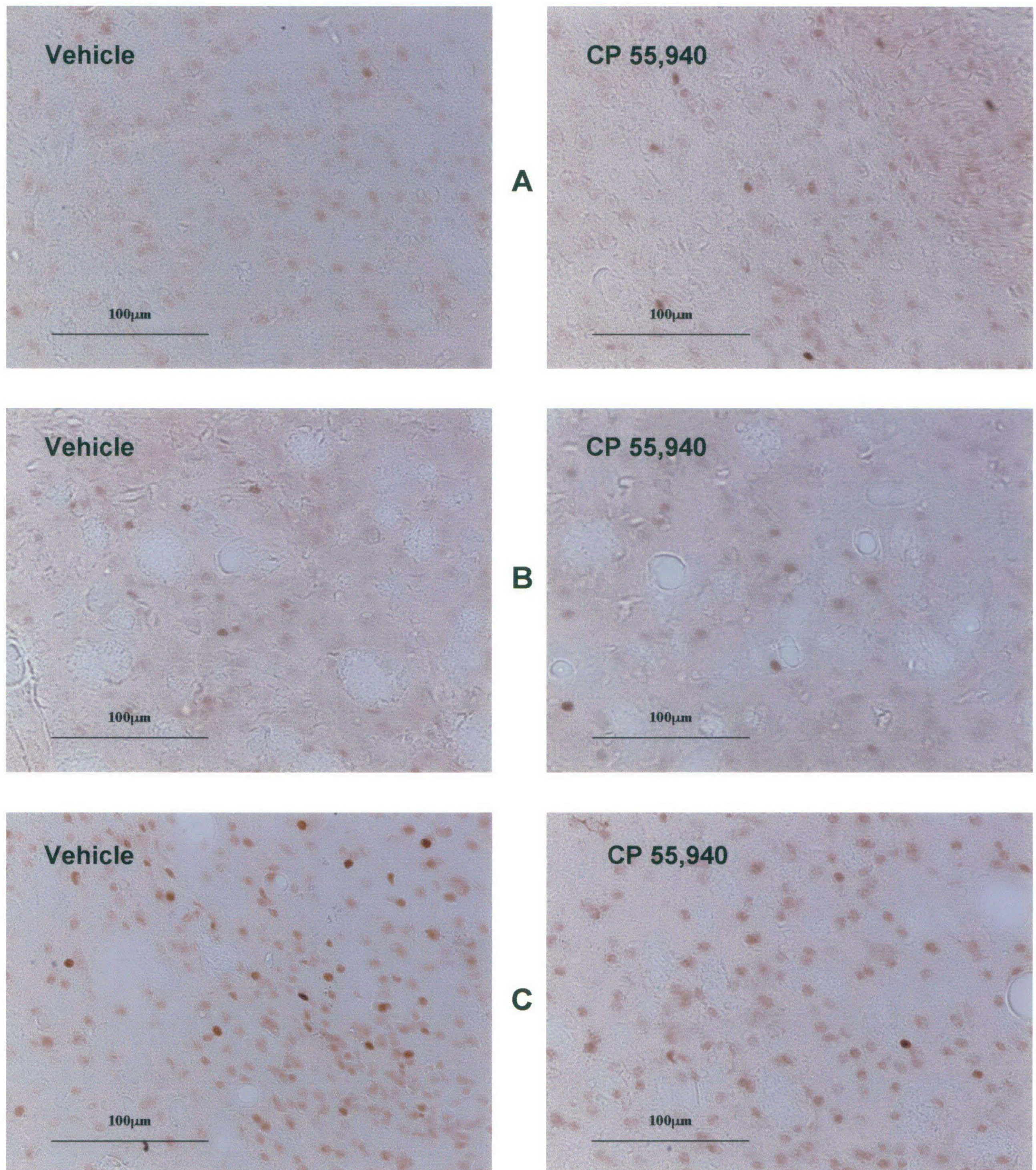

Figure 8.8 Photomicrograph of immunoreactive cells in the NAC shell for a) perinatal, b) adolescent, and c) adult groups. Higher Fos immunoreactivity was observed in adult groups compared to perinatal and adolescent groups.

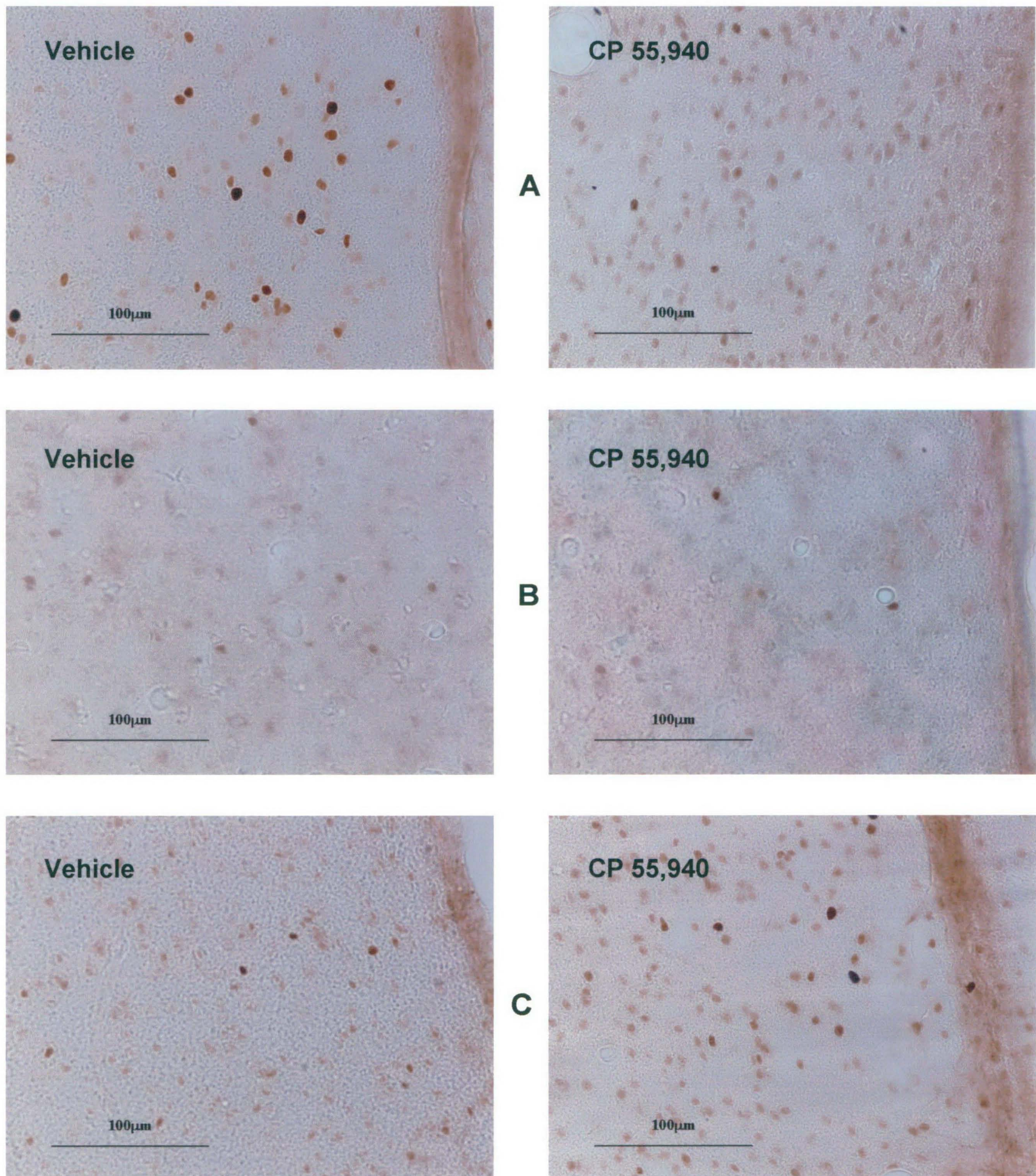

Figure 8.9 Photomicrograph of immunoreactive cells in the ventral LS for a) perinatal, b) adolescent, and c) adult groups. Higher Fos immunoreactivity was observed in perinatal groups compared to adult groups.

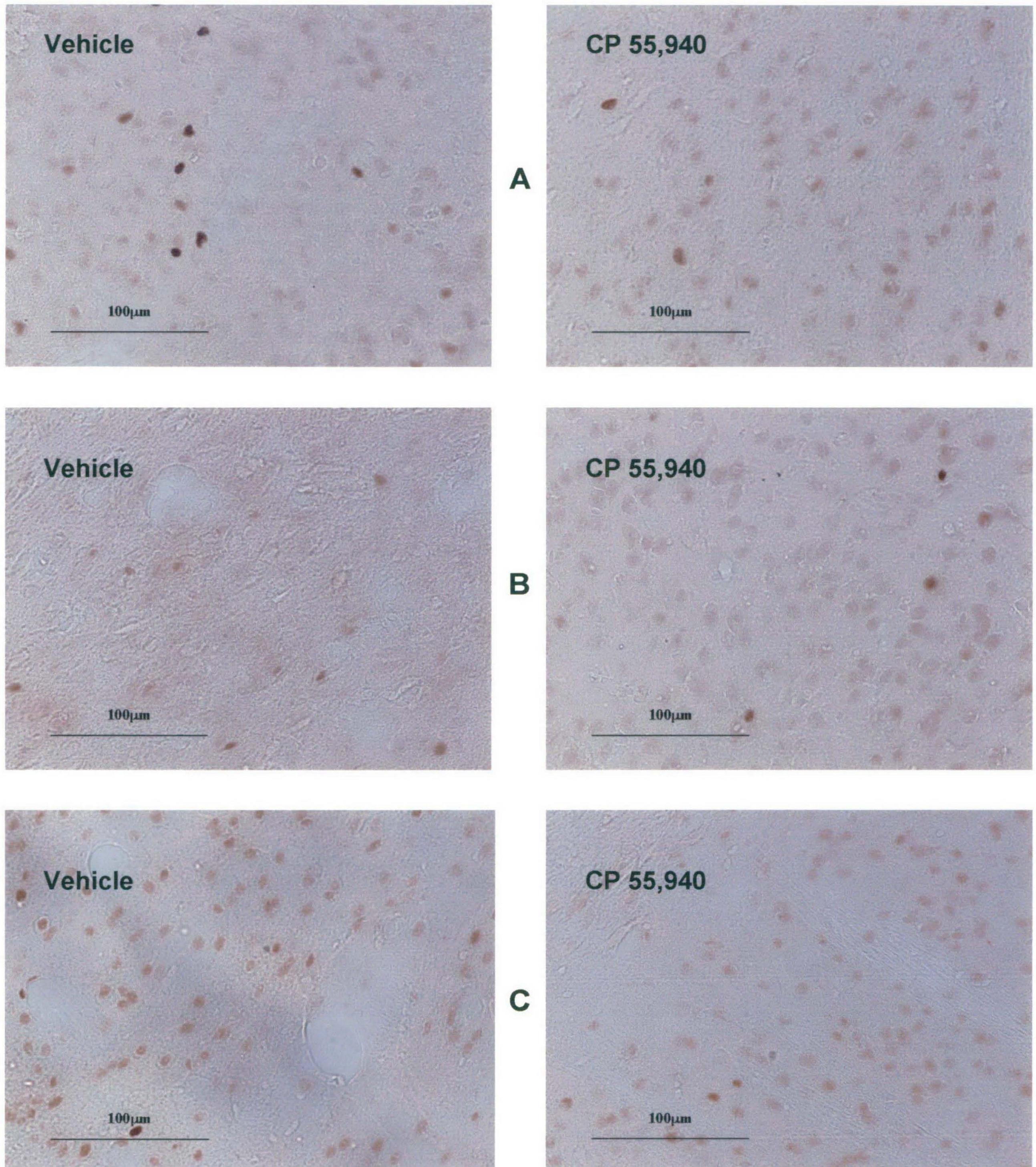

Figure 8.10 Photomicrograph of immunoreactive cells in the central nucleus of the amygdala for a) perinatal, b) adolescent, and c) adult groups. Higher Fos immunoreactivity was observed in perinatal groups compared to adolescent groups.

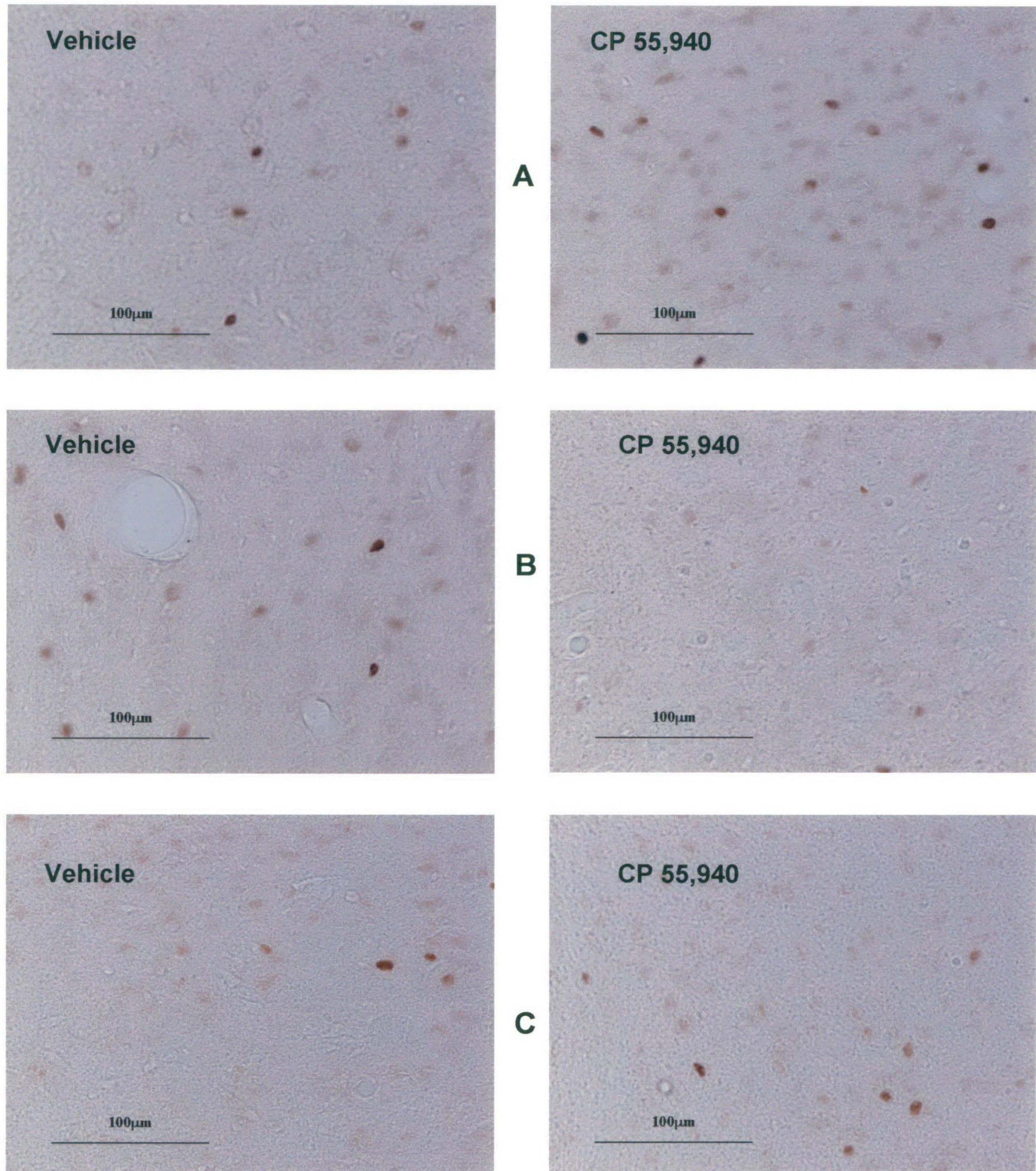

Figure 8.11 Photomicrograph of immunoreactive cells in the dorsolateral PAG for a) perinatal, b) adolescent, and c) adult groups. Higher Fos immunoreactivity was observed in perinatal groups compared to adult and adolescent groups.

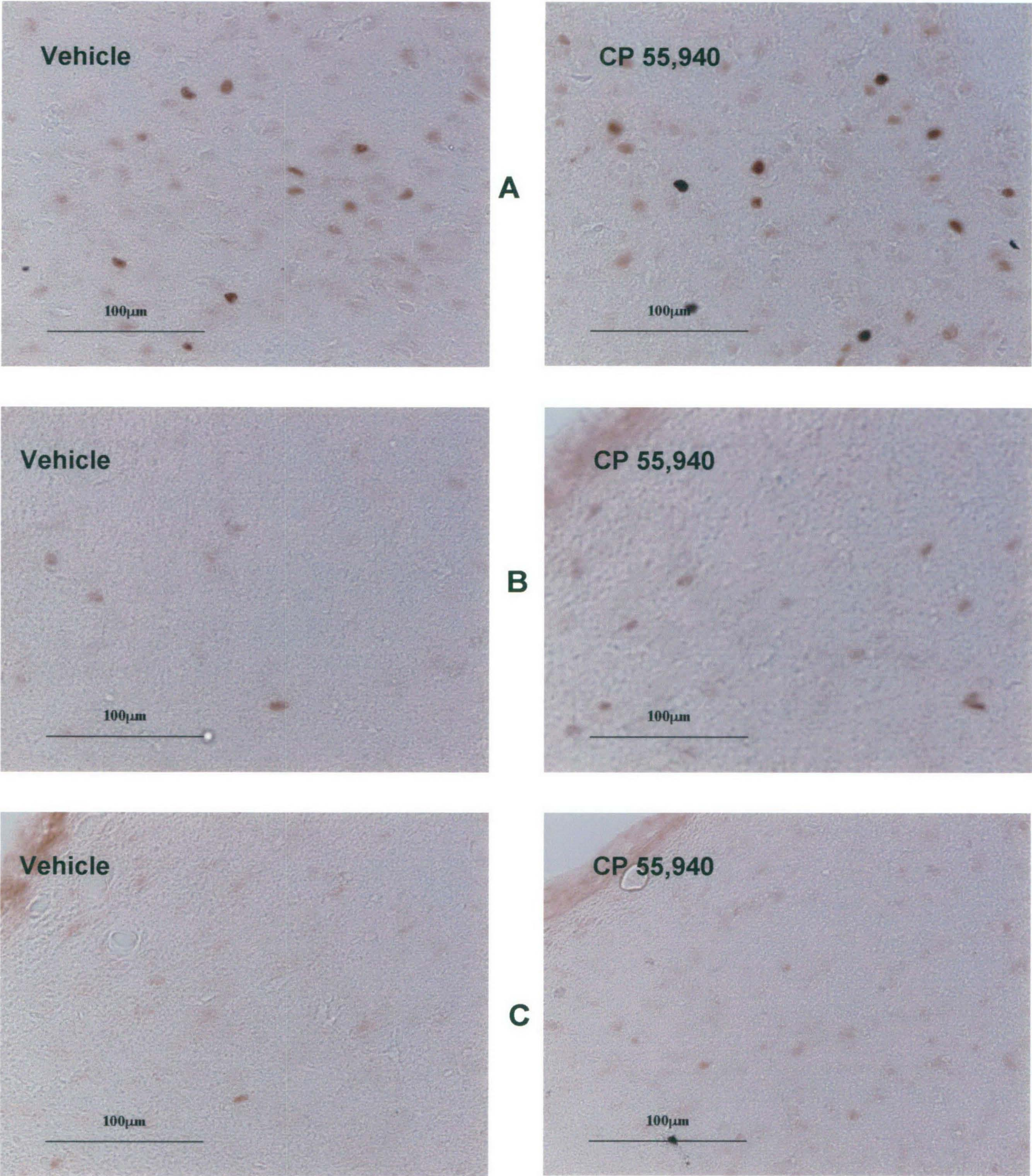

Figure 8.12 Photomicrograph of immunoreactive cells in the lateral PAG for a) perinatal, b) adolescent, and c) adult groups. Higher Fos immunoreactivity was observed in perinatal groups compared to adolescent and adult groups.

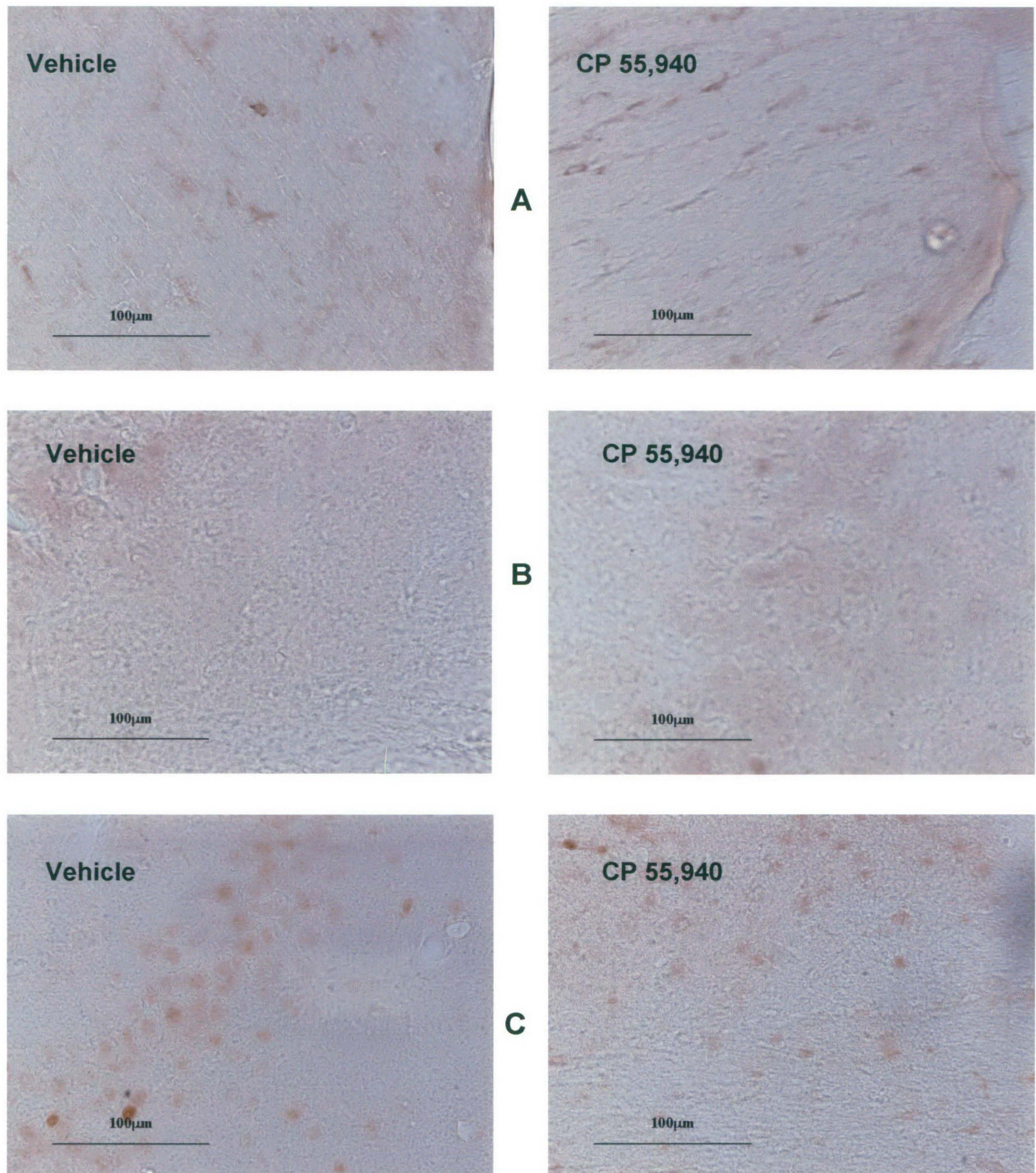

Figure 8.13 Photomicrograph of immunoreactive cells in the hippocampus CA3 for a) perinatal, b) adolescent, and c) adult groups. Higher *c-fos*-immunoreactivity was observed in perinatal groups, whereas the Fos exhibited by adolescent and adult groups was comparable.

8.5 Discussion

Previous work using *c-fos* immunohistochemistry identified lasting alterations of basal neuronal activity following perinatal (Singh et al., In press) and adult (Singh et al., 2005) onset cannabinoid exposure. However, the methods used in these previous studies could not rule out the possibility that altered basal *c-fos* expression may have reflected a change in brain activity due to a conditioned drug effect. The aim of the present study was to further examine basal *c-fos* expression in cannabinoid-exposed rats across a wider age range using confound-free methods. Following a 39-day drug-free period, *c-fos* immunohistochemistry was used to examine Fos-IR in several brain regions. The results can be summarised as follows: 1) differing baseline levels of Fos-IR expression dependent on age were observed in the piriform cortex, NAC shell, ventral LS, central and medial nucleus of the amygdala, dorsolateral and lateral PAG, and the CA3 region of the hippocampus; and 2) no long-term drug-induced alterations in basal neural activity were found between cannabinoid and vehicle groups at any ages. These results suggest that previous evidence (Singh et al., In press, 2005) of long-term cannabis-induced neuronal change using *c-fos* immunohistochemistry was likely due to a conditioned drug effect.

These previous findings (Singh et al., In press) using *c-fos* immunohistochemistry found widespread alterations in basal Fos-IR in several brain regions of adult rats exposed to THC during the perinatal period. A related study (Singh et al., 2005) involved the administration of THC to adult male rats (56-day old). Following a substantial drug-free period (55 days),

THC-treated rats showed increased basal levels of Fos-IR in several brain regions. One difference between the present study and our previous Fos-IR studies is that the former involved administration of heroin or its vehicle prior to perfusion. That is, we were primarily interested in examining the residual effects of THC exposure on heroin-induced Fos-IR. We nonetheless found that THC exposure altered the pattern of basal Fos-IR in control animals injected with vehicle only prior to perfusion. In our previous studies, a 2-h interval was used between injection and perfusion to permit the development of *c-fos* expression in response to the opioid challenge. It is therefore possible that the administration of vehicle to the rats after a long period of abstinence from THC may have induced a motivational state previously associated with THC administration (i.e., cues such as handling, injection, weighing). In the present study rats were anaesthetised and perfused immediately upon removal from their cages. Any conditioned drug effects would therefore not have the opportunity to alter *c-fos* expression.

Alternatively, the discrepant findings might simply reflect inherent differences between CP 55,940 and THC. For example, CP 55,940 is many times more potent than THC in producing behavioural effects. Although both drugs induce antinociception, hypothermia, hypoactivity, and catalepsy, THC produces these effects almost equipotently, while the locomotor activity suppressive effects of CP 55,940 are ten times more potent than its ability to induce catalepsy (Fan, Compton, Ward, Melvin, & Martin, 1994). Furthermore, perinatal exposure to THC alters adult corticosterone levels (Navarro et al., 1994), but CP 55,940 exposure in slightly older, yet still immature (i.e., peri-adolescent) rats, does not affect subsequent measures of

corticosterone levels (Biscaia et al., 2003). Further, as result of the affinity of THC for lipids, it tends to lodge in fat for long periods of time (Leighty, Fentiman, & Foltz, 1976), and has been detected up to 77 days after last drug administration in human cannabis users (Ellis, Mann, Judson, Schramm, & Tashchian, 1985).

An alternative explanation for the present results is that although *c-fos* is an accepted marker of neuronal activity, one of its limitations is that not all cells express the pathway leading to *c-fos* expression (Lawrence, Stroman, Bascaramurty, Jordan, & Malisza, 2004). The ability of a drug to induce Fos may also depend on the activating transmitter or secondary messenger. Consequently, *c-fos* expression may be absent even though the population of neurons is activated and capable of producing Fos (Hoffman, Smith, & Verbalis, 1993). Another limitation of this technique is that the range of stimuli that produces Fos-IR alteration in the CNS is largely undetermined (Suwanprathes, Ngu, Ing, Hunt, & Seow, 2003).

Then again, Fos immunohistochemistry might be unsuited to detecting drug-induced neuronal change following a substantial drug-free period. It is typically recommended that the time between the final drug administration and sacrifice be chosen to maximise Fos production. This period is typically estimated at 1-6 h after last drug administration, with baseline levels of Fos-IR typically returning by 24 h (Dragunow & Faull, 1989; Suwanprathes et al., 2003). Even so, it might be the case that this technique is better suited to the detection of long-term neural change when assessment occurs at the time of behavioural testing.

Nonetheless, the present findings are in good agreement with human imaging literature. For example, Tzilos et al. (2005) used magnetic resonance imaging (fMRI) to investigate the effects of cannabis on the morphology of the hippocampus in long-term heavy users. When compared to controls, cannabis users displayed no significant differences in volumes of gray matter, white matter, cerebrospinal fluid, or left and right hippocampus. Further, hippocampal volume in cannabis users was not associated with age at cannabis use onset or total lifetime use. These findings are consistent with recent literature suggesting that cannabis use is not associated with hippocampal change and structural changes within the brain overall. Conversely, around the same time this research was published, another study used Voxel-based fMRI (Matochik, Eldreth, Cadet, & Bolla, 2005) to compare long-term cannabis users to non-users. Cannabis users exhibited lower gray matter density in the right parahippocampal gyrus, and greater density bilaterally near the precentral gyrus and the right thalamus. Lower white matter density in the left parietal lobe and higher density around the parahippocampal and left side of the fusiform gyri were also exhibited. Further, a longer duration of cannabis use was found to be associated with higher white matter tissue density in the left precentral gyrus.

Presently, although drug-induced neuronal change was not found in cannabinoid-treated groups compared with controls, it is interesting to note that the number of Fos-labelled nuclei in adolescent treatment groups was lower than those of perinatal and adult treatment groups. Lower counts were exhibited in the piriform cortex, NAC shell, ventral LS, central and medial nucleus of the amygdala, dorsolateral and lateral PAG, and the CA3 region of

the hippocampus. The only difference being that perinatal rats exhibited the lowest hippocampal CA3 counts, and adult rats exhibited the lowest counts in the ventral LS. Clear differences in Fos-IR between adolescent and adult rats have been observed previously, with the adolescent rat brain exhibiting little or no Fos-IR in response to stress (Kellogg et al., 1998).

Perhaps the immunohistochemistry technique better detects naturally occurring changes in neuronal activity over time at different developmental ages, rather than being a suitable measure of residual drug-induced change. At the same time, it is often the case that rat behaviour does not always correlate with neuronal activation in relevant brain regions and subregions using the *c-fos* immunochemistry technique. For example, a previous study by Salchner et al. (2004) compared social interaction in young and old rats. Aged rats spent significantly less time (75%) in active social interaction than young rats indicative of increased anxiety. *C-fos*-immunohistochemistry was then used as a marker of neuronal activation. Lower Fos-IR was noted in aged rats compared to younger rats in the medial and basolateral amygdala, parvocellular region of the paraventricular hypothalamic nucleus, and the PAG (dorsomedial, dorsolateral, and ventrolateral). No differences in Fos-IR were observed in the hippocampus, septum or locus coeruleus. Thus, the observed increase in anxiety was not accompanied by *c-fos* expression in any of the key brain areas of the fear/anxiety circuitry known to be activated by anxiogenic stimuli.

However, a recent study (Gomez et al., 2003), found changes in the L1 gene associated with perinatal THC exposure in male rats. Most of the grey matter regions analysed, such as several cortical structures including the

basal ganglia, hippocampus, diencephalic regions and limbic regions, did not exhibit any change in their levels of L1 gene expression resulting from perinatal THC exposure, with the exception of the habenula and the septum nuclei, which showed modest increases. By contrast, perinatal THC exposure increased L1 gene expression in most of the white matter regions such as the fimbria, the stria terminalis, the stria medullaris, and the corpus callosum. Thus, it was concluded that it might be the case that the level of the protein expressed is in glial cells rather than on the neuronal L1. These findings suggest that neuronal change might be best detected on a micro rather than macro level, that the detection of change is highly dependent on the neurological measure used, and that neuronal change can occur in regions least anticipated in change.

Although there is growing behavioural evidence that cannabis use leads to lasting changes on cognitive domains such as working memory, learning, and emotion, these alterations are largely unsupported by drug-induced neuronal change. Overall, more definitive evidence is needed on the lasting effects of cannabinoid exposure on brain morphology and histology in both humans and animals, in order to conclusively determine whether cannabis administration can lead to neuronal changes that persist beyond the period of acute intoxication.

CHAPTER 9: GENERAL DISCUSSION

The main purpose of current research was to compare the lasting effects of cannabinoid exposure across various developmental periods. To the author's knowledge, this is the only cannabinoid research systematically investigating three major developmental ages (perinatal, adolescent, and young adult) at which cannabis exposure onset commonly occurs in humans. Another major aim of the present investigation was to compare the present findings on male rats to the author's previous research with females (O'Shea et al., 2004). This previous research found residual cognitive deficits (i.e., working memory and social anxiety) associated with adolescent, but not early adult onset cannabinoid exposure. To gain a broader understanding of both sex- and age-related cannabinoid effects, the current research also included an examination of perinatal drug-exposure. Furthermore, a broader range of behavioural measures was used, and an immunohistochemical examination of basal levels of *c-fos* immunoreactivity was included to determine whether any long-lasting drug-induced changes in patterns of neural activity could be detected.

An important feature of the current research was that perinatal cannabinoid administration took place during the early postnatal period, which mimicked third trimester pregnancy in humans. This consideration is important as most synaptic development occurs in third trimester pregnancy in humans, but in rats most synaptogenesis occurs in the first few days of postnatal life (Meyer & Kunkle, 1999). Most perinatal cannabinoid studies involve injecting the pregnant dam (for review see Abel, 1980). The administration of cannabinoids to pregnant rats may therefore bypass the

most critical period of development likely to inflict permanent changes in neurobehavioural function.

The effects of adolescent onset cannabinoid exposure were also examined. The investigation of adolescent onset cannabis use is an emerging area of research (Schneider & Koch, 2003, 2004). Human cannabis use is commonly initiated in adolescence (Scallet, 1991), which coincides with major neuronal changes in the CNS (Ehrenreich et al., 1999). Moreover, cannabis initiation appears to be occurring earlier in life compared to the recent past (Tonkin, 2002).

Early adult onset cannabinoid exposure was also examined. Some human studies (Ehrenreich et al., 1999; Pope et al., 2003) comparing early and late onset cannabis exposure in adolescence have found no cognitive deficits associated with exposure occurring at a later age. Likewise, animal studies involving adult rats support these findings (Deadwyler et al., 1995; Stiglick & Kalant, 1985; Nakamura et al., 1991). Further, direct adolescent-adult comparisons have shown cognitive deficits specific to adolescent onset exposure (O'Shea et al., 2004; Schneider & Koch, 2003).

9.1 Main Findings of the Current Research

In Chapter 3, learning was assessed in adult rats exposed to THC or its vehicle in the perinatal period using a double Y-maze. THC-treated rats performed similarly to controls in the spatial discrimination (reference memory) component of the maze in adulthood, yet significant delays in the acquisition of the delayed alternation component of the Y-maze (reference plus working memory) were observed. 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.

In Chapter 4, the cannabinoid-induced working memory deficits in Chapter 3 were further validated. Working memory (object recognition task) was assessed in adult rats exposed to CP 55,940 or its vehicle at perinatal, adolescent, or early adult ages. Object recognition was impaired by CP 55,940 treatment at all ages, suggesting an impairment of working memory regardless of age at onset of cannabinoid exposure. These findings (Chapter 3 and 4) suggest that cannabis exposure leads to deficits specific to working memory.

In Chapter 5, social anxiety (social interaction test) was assessed in adult rats exposed to CP 55,940 or its vehicle at perinatal, adolescent, or early adult ages. Social interaction was reduced by exposure to CP 55,940 at all ages, suggesting an increase in anxiety regardless of age at exposure.

In Chapter 6, the social interaction test data was further examined by assessing aggressive behaviours specifically. The results showed that rats treated with CP 55,940 at a perinatal age did not differ from controls. However, adolescent and adult CP 55,940-treated rats exhibited reduced aggressive behaviours in comparison with controls. These results suggest that cannabis exposure, despite cessation, might result in a slight, but measurable, decrease in aggression.

In Chapter 7, generalised anxiety (emergence test) was assessed in adult rats treated with CP 55,940 or its vehicle at perinatal, adolescent, or early adult ages. 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. These results suggest that cannabinoid exposure might lead to a slight decrease in anxiety-like behaviour in certain contexts.

In Chapter 8, *c-fos* immunohistochemistry was used to identify drug-induced differences in basal neural activity in rats exposed to CP 55,940 at perinatal, adolescent, and adult onset. In later adulthood, *c-fos*-immunoreactive cells were quantified in several brain regions. Differing baseline levels of *c-fos* expression dependent on age were observed in the piriform cortex, NAC shell, ventral LS, central and medial nucleus of the amygdala, dorsolateral and lateral PAG, and the CA3 region of the hippocampus. No significant drug-induced changes in basal neural activity were found between cannabinoid and vehicle groups at any ages. These results suggest that previous evidence of long-term cannabinoid-induced alterations of neuronal activity using *c-fos* immunohistochemistry may have been conditioned drug effects.

9.2 Implications of the Current Research

The current research suggests that ongoing investigation of lasting cannabinoid effects in males and females across species is an important avenue of investigation. Previous work (O'Shea et al., 2004) with females revealed that immature rats are more susceptible to cannabinoid-induced behavioural change than mature rats. The current results suggest that cannabinoid exposure in males might increase the possibility of maintaining long-lasting cognitive deficits, largely regardless of age at exposure. This is not to say that the theory of age-related exposure should be discounted.

Studies on adolescent versus adult male rats have suggested that younger male rats are most vulnerable to lasting cannabinoid effects (for example see Schneider & Koch, 2003). Nonetheless, sex-differences in cannabinoid effects remain to be fully determined, and future research might identify presently unknown mechanisms (e.g., fluctuations in brain chemistry that may serve to attenuate lasting cognitive deficits at transient points in time) that explain these differences.

The previous (O'Shea et al., 2004) and current research makes two important contributions to the literature on residual cannabinoid effects. First, these findings provide strong evidence that cannabinoid exposure at an immature age (prior to sexual maturity) leads to lasting cognitive deficits in later life such as deficits specific to learning, working memory and anxiety, so if cannabis exposure is initiated prior to young adulthood the likelihood of lasting cognitive deficits are greatly increased. Second, comparison of the previous and present research suggests that males are more sensitive than females to cannabinoid effects; therefore exposure even at a young adult age in males can lead to residual cognitive effects. The practical utility of the current animal research has implications for human cannabis use. That is, these data point to the dangers of habitual cannabis use in humans, particularly when the drug is taken early in life in both sexes, or used by males at any age.

9.3 Limitations and Future Directions for Research

CNS growth and development continues well past sexual maturity, and it is difficult to define at which point development ends (Smith, 2003). For example, in the present research, adolescent cannabinoid exposure took

place on PND 30, in agreement with other definitions of adolescence in the rat, i.e. 28-55 days of age (Ojeda & Urbanski, 1994), 28-60 days (Smith, 2003). Despite this consensus, it is not uncommon for the onset of sexual maturity to differ between males and females (i.e. as late as day 60 in the male) (Smith, 2003), and the magnitude and timing of these differences are species dependent (Sisk & Foster, 2004). Thus, these differences in themselves might impact on the results of studies addressing age at onset of drug exposure. The same applies to the human definition of adolescence, and as Spear (2002) has noted, the temporal boundaries of adolescence are elusive: in any species it is difficult to define when the first transition of adolescence begins to emerge and the last remnant persists.

More evidence of vulnerability to lasting cannabinoid effects at an early age is needed. For instance, a broader understanding of brain chemistry and behavioural development during adolescence is required to better characterise and understand susceptibility to drug exposure at this specific age (Smith, 2003). Early onset cannabis exposure at an adolescent age (<16 years old) has been associated with attention (Ehrenreich et al., 1999) and IQ (<17 years old) (Pope et al., 2003) deficits. Further, early onset users (<17 years old) have been observed to have smaller brains on the whole, a reduced percentage of cortical gray matter, a larger percentage of white matter volume, as well as higher CBF in males (Wilson et al., 2000). Interestingly, these findings agree with alcohol research (De Bellis et al., 2000) showing an association between early adolescent (mean age 17 years old) onset exposure and smaller hippocampal volume. At the same time,

these age-related effects are far from conclusive, and more solid and direct evidence of cannabinoid effects on early brain development is needed.

More evidence of cannabinoid effects on brain function is needed. In humans, existing imaging methods are not sensitive enough to detect cannabinoid-induced subcellular alterations (for review see Solowij, 1998), and in general, evidence of cannabinoid effects on the integrity of the brain are mixed. One study using fMRI (Tzilos et al., 2005) showed no evidence of hippocampal change in long-term heavy cannabis users. Conversely, a study using Voxel-based fMRI (Matochik et al., 2005) found that cannabis users exhibited brain irregularities such as lower gray matter density in the right parahippocampal gyrus, lower white matter density in the left parietal lobe, and higher density around the parahippocampal and left side of the fusiform gyri. In animals, some studies have failed to show THC-induced morphological changes such as cannabinoid receptor alterations (Westlake et al., 1991), but other studies have shown that cannabinoid exposure can lead to synaptic changes (Scallet et al., 1987), hippocampal change (Landfield et al., 1988), and alterations in neurotransmitter systems (Ali et al., 1989). These mixed findings suggest that it is yet to be fully determined whether cannabinoid exposure does indeed cause brain change.

More evidence of permanent cannabinoid-induced neurobehavioural change is required. Whilst the current behavioural studies largely suggest that cannabis exposure can lead to delayed learning, increased social anxiety, and a lasting impairment of working memory, it is premature to conclude that these effects are permanent. For example, in the double Y-maze, perinatal THC exposure was associated with measurable learning difficulties in

adulthood. However, as testing progressed the difference between groups narrowed, and by the final 3 days of the 25-day testing period, no significant differences between THC- and vehicle-treated groups were found. Thus, cannabinoid-induced neurobehavioural deficits might recover at later ages.

Continued research on the mechanisms underlying cannabinoid-induced neurobehavioural change is required. Much progress has been made in understanding the neural consequences of cannabinoid action. For example, perinatal cannabinoid exposure is associated with both alterations in cannabinoid receptor development, as well as effects on various neurotransmitters (e.g., dopamine, 5-HT, GABA, and opioid peptides) (Fernández-Ruiz et al., 1992; Fernández-Ruiz et al., 1997; Fernández-Ruiz et al., 1999; Fernández-Ruiz et al., 2000). Further, a great deal is now known about the cannabinoid system in general. For instance, the opioid and cannabinoid systems share direct correlates (Berrendero et al., 1999), and ANA functions as an endogenous agonist at both CB_1 and VR_1 receptors (Szallasi & Di Marzo, 2000). However, there is still ambiguity as to what these relationships represent, and why particular and multiple mechanisms are implicated in lasting change.

More information on cannabinoid dosing regimens is needed. In the human cannabinoid literature, the specification of what constitutes low, medium, and high cannabis use; intermittent or heavy frequent use; and dose per drug-taking session, is particularly broad (Jones, 1980). Likewise, cannabinoid dose and route of administration in animal studies also vary (for review see Abel, 1985). For example, in perinatal studies, it is not uncommon for cannabis extract dose to range from 4.2 mg/kg i.p. (Kawash et al., 1980) to

250 mg/kg s.c. (Gianutsos & Abbatiello, 1972). Further, it has been suggested that the lasting neurotoxic effects of THC appear to be specific to young rats (40-days old or less), when exposure is chronic (90 days+) (Scallet, 1991). However, in the previous (O'Shea et al., 2004) and current research, it was found that exposure for 21 days was sufficient to produce significant and lasting effects on working memory and social anxiety.

More information on sex-related cannabinoid effects is also needed. Few studies have examined cannabinoid effects in females of any species (Tseng & Craft, 2001). Studies in female animals are often performed after ovariectomy to eliminate potential oestrous cycle effects (e.g., Moreno et al., 2003) but the findings still agreed with previous research showing marked neurobehavioural cannabinoid effects in males, particularly dopamine receptor change (Fernández-Ruiz et al., 1992; Navarro et al., 1996). Further, it has been shown (through castration in male rats and ovariectomy in female rats) that hormones do not account for sex differences in dopamine receptor expression (Andersen, Thompson, Krenzel, & Teicher, 2002).

Interestingly, the current behavioural effects are similar to those produced by other recreational drugs. For example, working memory deficits in object recognition and lasting anxiety (elevated plus maze, social interaction and emergence tests) have been observed up to 3 months after MDMA treatment (Morley et al., 2001). Another study (McGregor et al., 2003) showed poorer memory in the object recognition task, depressive-like behaviour in the forced swim test, and increased anxiety-like behaviour in the social interaction and emergence tests, 8-18 weeks after MDMA administration. Recently (Clemens et al., 2004), anxiety-like behaviour in the social interaction test was observed

4 weeks after MDMA, METH, or combined MDMA/METH treatment. In further agreement with the current research, a recently published study (Koenig et al., 2005) showed that male rats exhibit higher sensitivity to repeated MDMA exposure than females. These similarities suggest that it would be of benefit to conduct systematic comparisons of residual effects across a broader range of recreational drugs.

9.4 Conclusions

The present behavioural findings suggest that cannabinoid exposure throughout all periods of life may compromise cognitive abilities that influence learning, working memory, and social behaviour. These findings also point to possible sex differences, which warrant further study. Although these behavioural deficits were not accompanied by changes in *c-fos* expression, a common issue concerning cannabinoid research is that drug-induced changes in brain morphology and function have yet to be fully determined. Additionally, both the age- and sex-dependent effects of cannabinoid exposure are particularly vexing at this point in time.

The mechanisms underlying the current behavioural findings can only be surmised, but one of many possibilities is that cannabinoid-induced behavioural change might partly be explained by dopamine-CB_1 receptor interactions. Currently, it is fairly well established that cannabinoids can induce alterations in dopaminergic neurons (i.e., decreased binding), particularly when onset of exposure occurs in early life (Rodríguez de Fonseca et al., 1992). Further, there is evidence of cannabinoid binding sites in the brain from an early postnatal age (Rodríguez de Fonseca, Ramos, Bonnin, & Fernández-Ruiz, 1993), and that these CB_1 receptors might down-

regulate, as shown following adult onset cannabinoid exposure (Rodríguez de Fonseca, Gorriti, Fernández-Ruiz, Palomo, & Ramos, 1994). Although the relationship between CB_1 and dopamine receptors are not well understood, it is likely that cannabinoid exposure might disrupt normal dopaminergic functioning, and thus permanently impede the modulatory relationship between the dopamine and cannabinoid systems (Moreno et al., 2003). Further, sex differences in CB_1 receptor binding have been observed in the striatum and ventral mesencephalon but not the limbic forebrain (Rodríguez de Fonseca et al., 1993), perhaps partially explaining the sex-dependent neurobehavioural effects of cannabinoids (Navarro, Rubio, & Rodríguez de Fonseca, 1995). Likewise, naturally occurring sex-related differences in D_1 receptors (increases of 65% in males and 35% in females) and D_2 receptors (144% increase in males and 31% in females) have been observed at an adolescent age in the rat brain; and by adulthood, dopamine production in males was still higher than that of females (Andersen, Rutstein, Benzo, Hostetter, & Teicher, 1997). At the same time, an important feature of cannabinoid action is its influence on multiple systems of the organism--the role of different neurotransmitter systems in mediating the effects of cannabinoids has been well studied employing a variety of methods, however, the resulting findings are contradictory (Machula, Dudkin, & Barkov, 1992). Recently, it has been suggested that the L1 cell adhesion molecule is implicated in cannabinoid-induced neurobehavioural alterations (Gomez et al., 2003). Investigation of this protein in the human brain suggests that L1 is temporally and spatially expressed in the developing brain, and may play important roles in neural cell migration, neurite elongation, and axonal

fasciculation (Tsuru, Mizuguchi, Uyemura, & Takashima, 1996). A recently published study found that chronic THC exposure leads to up-regulation of the brain-derived neurotrophic factor (BDNF), which is known to modulate synaptic plasticity and adaptive processes underlying memory (Butovsky et al., 2005).

In conclusion, the current thesis adds to literature on the neurobehavioural consequences of cannabinioid exposure. It not only highlights the lack of animal research on residual cannabinoid effects, but also illustrates that much more human research on this important health issue is needed. In sum, the present research established that both THC and CP 55,940 exposure can lead to lasting behavioural deficits (learning, working memory, and social behaviour). However, the current findings alone are not sufficient to determine whether these residual deficits are permanent. Nonetheless, there is growing evidence that cannabis exposure, from the perinatal period through to adulthood, may lead to lasting and potentially permanent cognitive deficits.