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ORIGINAL INVESTIGATION

Miguel Biscaia · Susana Marín · Beatriz Fernández · Eva M. Marco · Marina Rubio · Carmen Guaza · Emilio Ambrosio · Maria Paz Viveros

Chronic treatment with CP 55,940 during the peri-adolescent period differentially affects the behavioural responses of male and female rats in adulthood

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Abstract *Rationale:* Despite the increasing use of cannabis among adolescents, there is scarce information about the long-term effects of cannabinoid receptor agonists in appropriate animal models. *Objectives:* We aimed to investigate the behavioural features of adult male and female Wistar rats that had been exposed to a chronic treatment with the cannabinoid receptor agonist CP 55,940 (CP) during the juvenile period. *Methods:* CP (0.4 mg/kg i.p.) or its corresponding vehicle was administered once daily, from day 35 to day 45. In adulthood, the animals were tested in the holeboard, the open field and the elevated plus-maze, under different stress (illumination) conditions. After a resting period, the serum corticosterone levels (radioimmunoassay) of the animals were measured. The effects of CP on food intake and somatic growth were monitored throughout the experimental period. *Results:* The CP treatment induced significant sex-dependent effects on holeboard activity, as well as a decrease in the level of emotionality/anxiety in the open field and in the plus-maze. The animals receiving CP also showed diminished food intake and body weights during the treatment period, but both parameters recovered normal values during the period after treatment. No significant effect of the CP treatment on corticosterone levels was found. *Conclusions:* The results demonstrate that chronic administration of CP during the peri-adolescent period resulted in marked behavioural effects in adulthood. The nature of these effects depended on the sex of the animals and on the specific behavioural test. The possible neurobiological substrates underlying the effects of CP are discussed.

Keywords Rat · CP 55,940 · Juvenile period · Long-term effects · Holeboard · Open field · Plus-maze

M. Biscaia · B. Fernández · E. Ambrosio
Departamento de Psicobiología de la UNED,
Madrid, Spain

S. Marín · E. M. Marco · M. Rubio · M. P. Viveros (✉)
Departamento de Fisiología (Fisiología Animal II),
Facultad de Biología, Universidad Complutense,
28040 Madrid, Spain
e-mail: pazviver@bio.ucm.es
Tel.: +34-91-3944993
Fax: +34-91-3944935

C. Guaza
Instituto Cajal,
Madrid, Spain

Introduction

Cannabis is the most widely used illicit drug in many western countries. The acute effects of cannabinoid receptor agonists have been well characterised in adult rodents, and they include effects on motor function and anxiety, hypothermia, analgesia and stimulation of adrenocortical activity (Rubino et al. 1994; Chaperon and Thiebot 1999; Fuentes et al. 1999; Manzanares et al. 1999a; Sañudo-Peña et al. 2000; Berrendero and Maldonado 2002; Marín et al. 2003). There is also evidence about the behavioural, neurochemical and neuroendocrine consequences of pre- and perinatal exposure to either cannabis preparations or to its main psychoactive component, Δ^9-tetrahidrocannabinol (THC), in rats (Kumar et al. 1990; Navarro et al. 1994, 1995; Vela et al. 1998; Fernández-Ruiz et al. 1999). However, despite the increasing use of cannabis among adolescents (Gruber and Pope 2002), there is scarce information about the effects of cannabinoid receptor agonists in appropriate animal models during the juvenile period. Previous data obtained from both experimental animals (Vela et al. 1998; Manzanares et al. 1999b) and humans (Lynskey et al. 2003) indicate that cannabinoids might initiate the consumption of opiates and other drugs of abuse. In the present study, we aimed to address the behavioural features of adult rats that had been exposed to a chronic treatment with CP 55,940 (CP) during the juvenile period. For this purpose, we investigated the effects of a daily administration of the drug from 35 days to 45 days of age, on a wide range of behavioural parameters that were recorded in adulthood. We used a battery of tests that

provide complementary data about diverse aspects of the spontaneous behaviour of the animals and their anxiety-related responses. The holeboard provides independent measures of motor activity and site-directed exploration, and the elevated plus-maze test has been validated for the evaluation of anxiety in rodents (Pellow et al. 1985; File 1992). We have also used an illuminated open field. This test provides additional information about emotional reactivity and locomotor activity (Gray 1987; De Cabo et al. 1995). The change in the illumination conditions with respect to the holeboard and the plus-maze (which were performed under red light) allows the recording of behavioural responses under different intensities of stress. In order to address the possible existence of sex differences, we used rats of both sexes. We also determined the serum corticosterone levels of the animals as a possible correlate of their behavioural state. To assess possible effects of CP on food intake (F.I.) and somatic growth, chow consumption and body weights (B.W.s) were monitored throughout the experimental period. This study provides the first evidence for long-term sex-dependent behavioural effects of a chronic treatment with CP in young rats.

Materials and methods

Animals, experimental conditions and pharmacological treatments

We used Wistar albino rats of both sexes. Subjects were the offspring of rats purchased from Harlan Interfauna Ibérica S.A. (Barcelona, Spain) which were mated (one male × two females) in our laboratory approximately 3 weeks after their arrival. All animals were maintained at a constant temperature (20±1°C) and in a reverse 12-h/12-h dark/light cycle (lights on at 0635 hours), with free access to food (commercial diet for rodents A04/A03; Panlab, Barcelona, Spain) and water. On the day of birth (postnatal day 0), litters were sex-balanced and culled to 10±1 pups per dam. The animals were weaned at 22 days of age. CP 55,940 (CP) (Tocris) (0.4 mg/kg) or its corresponding vehicle (VH) [ethanol:cremophor:saline (1:1:18) (cremophor, Fluka BioChemiKa)] was administered i.p., once daily, from day 35 to day 45 (11 injections), at a volume of 2 ml/kg. The dose of CP was chosen on the basis of pilot experiments in our laboratory and our previous study on acute effects of CP in 40-day old rats (Romero et al. 2002). All experimental procedures were carried out between 0800 hours and 1430 hours. The experiments performed in this study are in compliance with the Royal Decree 223/1988 of 14 March (BOE 18) and the Ministerial Order of 13 October 1989 (BOE 18) about protection of experimental animals, as well as with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The "Principles of laboratory animal care" (NIH publication no. 85–23, revised 1985) were followed.

Behavioural testing

Behavioural testing started at 75 days of age and was completed within 9 days. On the day of testing, the animals were habituated in a quiet laboratory for a 30-min period, before experimental procedures began. Animals performed each test individually. As described in previous work (De Cabo et al. 1995; Viveros et al. 2001), the order of testing was as follows: holeboard, open field, plus-maze. We allowed an interval of 3 days among the three tests for recovery of the animals from the previous testing conditions.

Holeboard test

The holeboard was a box (60×60×45 cm) with matte-painted metallic walls and a plastic-covered wooden floor bearing four equally spaced holes (3.8 cm in diameter) and divided into 36 squares (10×10 cm). The duration of the test was of 5 min, and it was performed under red light. The parameters measured were: external ambulation (EA; number of line crossings in the periphery, by the walls), internal ambulation (IA; number of line crossings in the remainder area), frequency and duration (s) of head-dipping, frequency of rearings (number of times that the animal stood on its rear limbs), frequency of facial grooming and defecation (number of boluses). We also estimated the percentage of internal ambulation to total (external + internal) ambulation (% IA).

Open field test

The open-field arena consisted of a cylinder (75 cm diameter × 50 cm high) with a floor divided into 19 sections of a similar area by two concentric circles (17 cm and 45 cm diameter) and a series of radii, and was made of the same materials mentioned earlier. The duration of the test was 3 min. A white-light lamp of 100 W was placed 80 cm over the centre of the arena during testing. The parameters measured were: EA (number of squares adjacent to the wall entered with the four limbs), IA (number of squares in the central area entered with at least three limbs), frequency of rearing, frequency of facial grooming, immobility [duration (s) of periods without activity, although movement of whiskers could be shown], and defecation. We also estimated the percentage of IA to total (external + internal) ambulation (% IA).

Plus-maze test

The plus-maze consisted of two open arms (50×10 cm) and two enclosed arms of the same size with 40-cm-high walls arranged so that the arms of the same type were opposite to each other. The junction of the four arms formed a central square area (10×10 cm). The apparatus was made of hard plastic material and elevated to a height of 62 cm. The test was carried out for 5 min under red light. The measures recorded were frequency and duration (s) of arm visits, separately for open and closed arms. An arm was considered to be visited when the animal entered it with the four limbs. We also estimated the total number of entries and total time spent in both types of arms, the percentage of entries to the open arms to the total number of entries, and the percentage of time spent in the open arms to the total time in arms.

Each test was started by placing the animals in the area of the apparatus considered more behaviourally neutral [one of the corners (holeboard), an external square (open field), at the centre of the apparatus facing one of the enclosed arms (plus-maze)] so that the animal was not artificially induced to perform a significant pattern (De Cabo et al. 1995; Viveros et al. 2001). The three apparatus were thoroughly cleaned at the end of every test.

Corticosterone assay

Before the sacrifice of the animals for corticosterone determinations, we allowed a period of recovery from the last behavioural test (plus-maze). All males were sacrificed at 90 days of age, whereas females were sacrificed between 90 days and 95 days of age when they were in oestrus (oestrus cycle controlled by vaginal smears). The animals were killed by decapitation. Blood samples were collected from the trunk and centrifuged (3000 rpm for 15 min), and serum was stored at −80°C. Corticosterone was measured using a solid-phase ^{125}I radioimmunoassay (Coat-A Count Rat Corticosterone kit, Diagnostic Products Corp., Los Angeles, CA). The detection limit was 5.7 ng/ml and the intra-assay and inter-assay coefficients of variation were less than 10%.

Food intake and body weight

F.I. and B.W.s were monitored throughout the experimental period. Chow consumption was recorded according to a previously described method (De Cabo and Viveros 1997). A measured amount of chow (250 g) was provided to every cage at the beginning of the dark phase of the cycle. Each cage housed 5±1 animals of the same gender and treatment condition. Chow was measured again 24 h later and the differences between the first and the second days were calculated. Amounts consumed per animal per day were averaged by dividing the calculated data by the number of animals per cage. Therefore, at all evaluations, the F.I. value assigned to each individual was its cage average, as previously described (De Cabo and Viveros 1997). To avoid the possible interference of F.I. before treatment varying between treatment groups with the actual effect of treatment, we calculated the increments of F.I. and B.W.s considering day 30 as the point of reference. For F.I. we calculated the increment (Δ) in grams per animal at each time point, as the difference between F.I. at a given day (day t) and F.I. at day 30, i. e. $\Delta\ grams_t/animal = F.I._{-t} - F.I._{30}$. The same criterion was followed to calculate the increment in B.W.: $\Delta\ grams_t/animal = B.W._{-t} - B.W._{30}$. The corresponding values ($\Delta\ grams_t/animal$) were employed for the statistical analysis. In order to evaluate the short- and long-term effects of the pharmacological treatment, we analysed the data corresponding to the period of treatment (days 35–45) as well as the data obtained at a later stage, after treatment (days 52–89).

Statistical analysis

Behavioural and endocrine (corticosterone levels) data were analysed by two-way (ANOVA), with the two factors being sex and pharmacological treatment. The data were previously tested for normality (Kolmogorov-Smirnov test) and equal variance (Levene median test). In those cases in which the data did not fulfil these criteria, we used a rank transform before performing the ANOVA. The data from F.I. and B.W.s were analysed using a stepwise regression model. The LSD test with a level of significance set at $P<0.05$ was used for post-hoc comparisons.

Results

Holeboard test

The results obtained in the holeboard test are represented in Table 1. With respect to EA, the ANOVA showed significant overall effects of both the pharmacological treatment ($F_{1,50}=12.5$, $P<0.001$) and sex ($F_{1,50}=30.6$, $P<0.001$), as well as a significant interaction between these two main factors ($F_{1,50}=8.4$, $P<0.01$). Post-hoc comparisons indicated that the chronic CP treatment significantly reduced EA in females, whereas no significant differences were found between control and CP treated males. Whether or not the females received CP, they showed a significantly increased EA when compared with males. The two-way ANOVA did not reveal any significant effect of the pharmacological treatment for IA or for the % IA. However, a significant overall effect of sex on % IA was found ($F_{1,50}=8.2$, $P<0.01$). In view of this sex difference and the mean values appearing in Table 1 for this parameter, we performed additional separate one-way ANOVAs for the two sexes. As expected, the analysis revealed that CP induced a significant increase in the % IA of females ($F_{1,20}=5.96$, $P<0.05$). For rearing frequency, the ANOVA revealed a significant effect of sex ($F_{1,50}=17.7$, $P<0.001$) and a significant interaction between sex and pharmacological treatment ($F_{1,50}=10.6$, $P<0.01$). The LSD test showed that the chronic CP treatment significantly decreased the rearing behaviour in females, whereas it did not affect this parameter in males. Besides, the sex differences only affected the control animals, with females showing an increased rearing frequency. Significant interactions between sex and pharmacological treatment were found for both, head-dipping frequency ($F_{1,50}=12.2$, $P=0.001$) and head-dipping duration ($F_{1,50}=17.2$, $P<0.001$). Post-hoc comparisons showed that the CP treatment induced significant decreases in both exploratory parameters in males. However, in females, the pharmacological treatment only induced a significant effect (an increase) in head-dipping duration. With respect to sex differences, control females showed lower values in the two exploratory parameters when compared with control males, whereas the opposite effect was found in the CP-treated animals. No significant differences were found for grooming frequency, whereas the ANOVA revealed a significant overall effect of sex on defecation score ($F_{1,50}=15$, $P<0.001$), with females showing the lowest values.

Table 1 Effects of chronic CP 55,940 treatment on hole board activity. Values represent the mean±SEM from the number of animals indicated in parenthesis

	External ambulation (line crossing)	Internal ambulation (line crossing)	%Internal ambulation	Rearing frequency	Head dipping frequency	Head dipping duration (s)	Grooming frequency	Defecation (no. of boluses)
Males								
Control (16)	86.3±5.6	47±3.9	35.3±2.5	19.8±1.5	12.6±1	28.7±4.2	1.6±0.5	5.4±1.1
CP55,940 (16)	81.2±6.1	42.1±3.8	34.7±2.9	25.3±2.1	8.3±0.6*	13.9±1.0*	2.5±0.6	6.8±0.6
Females								
Control (13)	153.5±11.4#	42.7±3.3	23±2.4	38.8±3.2#	9.5±1.2#	18.3±3.2#	2±0.5	2.2±0.7
CP55,940 (9)	102.2±7.6*#	46.4±4.3	31.4±2.2*	27.7±3.6*	12.8±1.7#	29.1±6.5*#	1.3±0.3	2.8±1.1

* LSD Test: $P<0.05$ vs the corresponding control group
# LSD Test: $P<0.05$ vs the corresponding male group; see text for significant overall effects of sex

Table 2 Effects of chronic CP 55,940 treatment on open field activity. Values represent the mean±SEM from the number of animals indicated in parenthesis. See text for significant overall effects of sex

	External ambulation (squares entered)	Internal ambulation (squares entered)	%Internal ambulation	Rearing frequency	Grooming frequency	Immobility time (s)	Defecation (no. of boluses)
Males							
Control (16)	55.8±4.0	11.5±1.7	16.2±2.3	12.2±1	0.6±0.2	14.7±4.3	6±0.7
CP 55,940 (17)	47.8±4.6	13.1±1.8	21.5±2.3	14.5±1.7	1.2±0.4	10.0±3	5.9±0.5
Females							
Control (13)	67.4±4.1	16.7±2	19.6±1.8	21.7±2.8	1±0.3	2.1±1	3.5±0.7
CP 55,940 (10)	61.5±3.8	19.8±3.2	23.2±2.2	17.9±2.6	0.7±0.4	6.9±2.3	4.1±0.6

Open-field test

The results obtained in the open field test are represented in Table 2. No significant effects of the pharmacological treatment or interaction between factors were found for any of the parameters measured in this test. However, the animals treated with CP showed an increased %IA, with this trend being in the limit of the statistical significance ($F_{1,52}$=3.9, P=0.055). Significant overall effects of sex were found for EA ($F_{1,52}$=8.3, P<0.01), IA ($F_{1,52}$=8.1, P<0.01) and rearing frequency ($F_{1,52}$=8.1, P<0.01), as well as for immobility time ($F_{1,52}$=5.6, P<0.05) and defecation score ($F_{1,51}$=10.9, P<0.01). As Table 2 shows, females showed increased values for EA, IA and rearing frequency, and decreased values for immobility time and defecation. The analysis of the effect of CP in each sex (one-way ANOVA) showed that the increased immobility time of the CP-treated females was in the limit of the statistical significance ($F_{1,21}$=4.3, P=0.051).

Plus-maze test

The results obtained in the plus-maze test are represented in Fig. 1. The ANOVA performed on the percentage of entries in the open arms revealed a significant overall effect of sex ($F_{1,44}$=5.3, P<0.05), whereas no significant effect of the CP treatment was found for this parameter (Fig. 1A). With respect to the percentage of time in the open arms, the ANOVA showed a significant effect of the pharmacological treatment ($F_{1,44}$=8, P<0.01), with the CP-treated animals showing the highest value. The ANOVA also revealed a significant overall effect of sex ($F_{1,44}$=6.2, P<0.05), with females showing the highest values (Fig. 1B). No significant effect of either the pharmacological treatment or sex was found for the number of entries in the closed arms (Fig. 1C). No significant interaction between factors was found for any of the parameters measured in this test.

Corticosterone assay

The analysis of the data from corticosterone determinations showed a significant effect of sex with females showing the highest corticosterone levels ($F_{1,44}$=56.7, P<0.001). No significant effect of the CP treatment or interaction between factors was found. Mean (ng/ml)±SEM: VH males, 505.4±56.4 (n=16); CP males, 484.1±40.6 (n=17); VH females, 916.3±83.9 (n=8); CP females, 1026.6±70 (n=7). These data correspond to the females in which we observed a clear oestrus. The analysis including additional females in which other phases of the oestrus cycle were observed rendered similar results (data not shown).

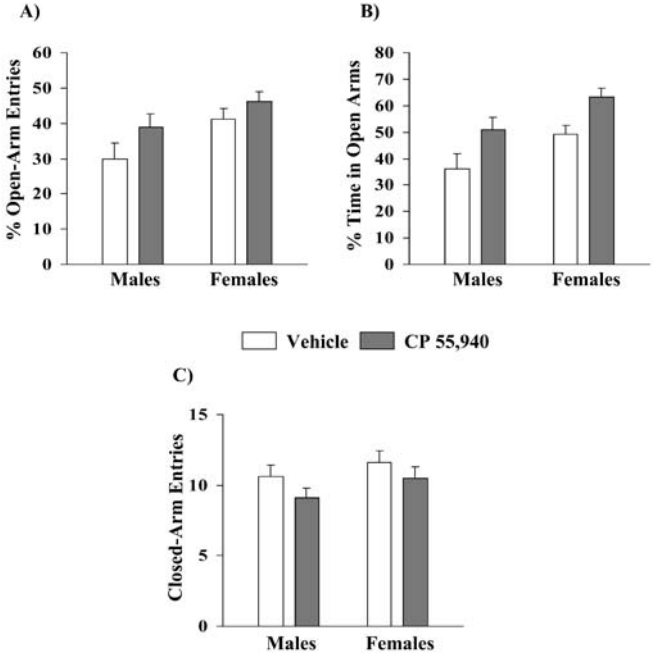

Fig. 1A–C Effects of chronic CP 55,940 (*CP*) treatment on plus-maze activity. The following results are shown: **A** percentage of open-arm entries; **B** percentage of time in open arms; **C** closed-arm entries. CP (0.4 mg/kg) or its corresponding vehicle (*VH*) (see text) was administered once daily, from day 35 to day 45. In adulthood (starting at 75 days of age), the animals were tested in the holeboard, the open field and the plus-maze. We allowed an interval of 3 days among the three tests for recovery of the animals from the previous testing conditions. Histograms represent the mean±SEM from the following number of animals: males: VH (13), CP (17); females: VH (8), CP (10). See text for significant overall effects of pharmacological treatment and sex

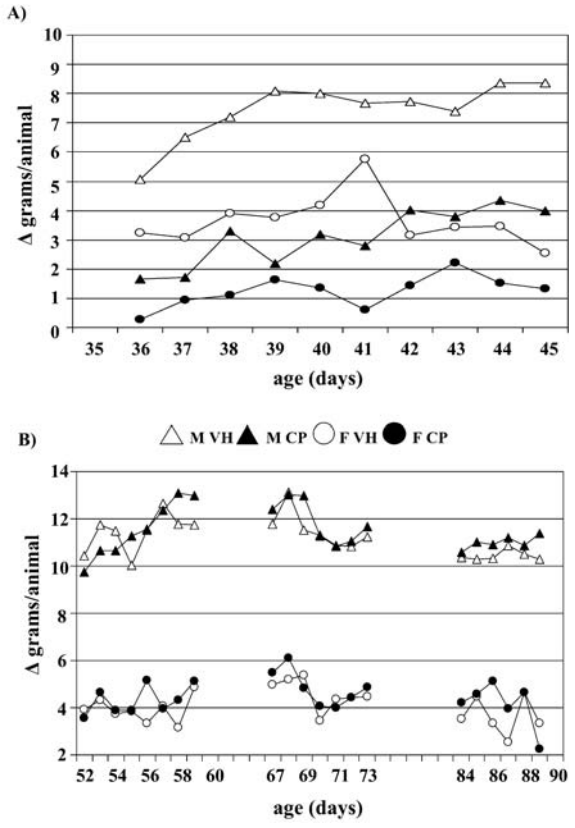

Fig. 2A, B Effects of chronic CP 55,940 (*CP*) treatment on food intake. **A** During treatment, days 35–45, CP (0.4 mg/kg) or its corresponding vehicle (*VH*) (see text) was administered once daily. **B** After treatment, days 52–89. The food intake value assigned to each individual was its cage average (5±1 animals per cage, 2–3 cages per experimental group). Values represent the increment (Δ) in grams consumed per animal at each time point (day t), considering day 30 as the point of reference (see Methods). *M* males, *F* females. See text for significant differences between groups

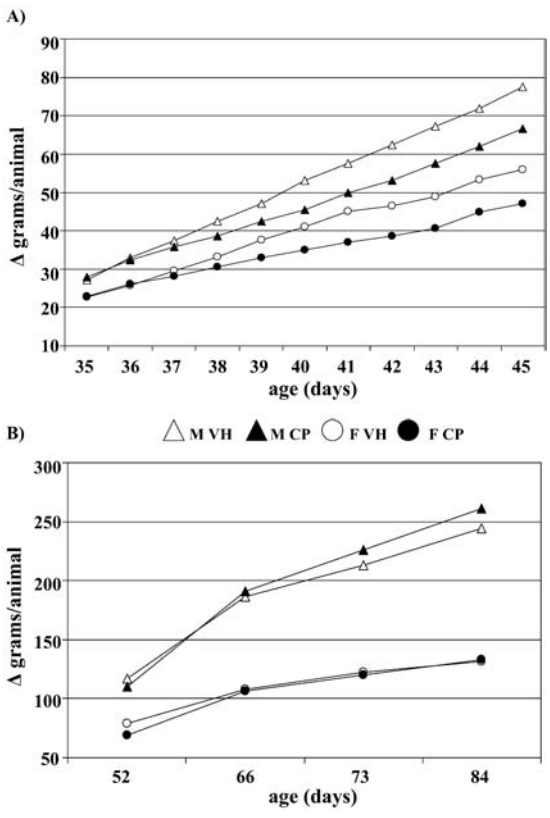

Fig. 3A, B Effects of chronic CP 55,940 (*CP*) treatment on somatic growth. **A** During treatment, days 35–45, CP (0.4 mg/kg) or its corresponding vehicle (*VH*) (see text) was administered once daily. **B** After treatment, days 52–84. Each animal was weighed individually. Number of animals per experimental group: males (*M*): VH (16), CP (17); females (*F*): VH (13), CP (10). Values represent the increment (Δ) in body weight (*g*) per animal at each time point (day t), considering day 30 as the point of reference (see Methods). See text for significant differences between groups

Food intake and body weight

The results obtained from the recording of F.I. and B.W.s are represented in Fig. 2 and Fig. 3. The analysis of the data from F.I. during the period of treatment with CP showed significant overall effects of the pharmacological treatment (t=−5.1, $P<0.001$) and sex (t=−2.4, $P<0.05$), as well as significant interactions sex × treatment (t=−3.1, $P<0.01$), and days × sex (t=3.7, $P<0.001$). As Fig. 2A shows, the CP treatment significantly decreased F.I. during this period, and the effect was more marked in males than in females. Regarding the sexual differences, F.I. was higher in males than in females, with this difference being significant (LSD test) on day 44 and day 45. During the period after treatment, the analysis only detected a significant overall effect of sex on F.I. (t=25.9, $P<0.001$), with males showing an increased F.I. irrespective of the pharmacological treatment (Fig. 2B). With respect to B.W.s, the analysis of the data from the period of treatment rendered significant effects of the three main factors, pharmacological treatment (t=4.6, $P<0.001$), sex (t=−5.5, $P<0.001$) and days (t=17.3, $P<0.001$), as well as significant interactions days × treatment (t=−5.2, $P<0.001$) and days × sex (t=6.9, $P<0.001$). The LSD test indicated that the CP treatment significantly decreased the B.W.s from day 40 and until the end of the treatment. Besides, the sex differences (males increased B.W.s) began to be significant on day 39 and were more marked in the following days (Fig. 3A). During the period after treatment, the analysis of B.W.s indicated significant effects of sex (t=−5.9, $P<0.001$) and days (t=10.5, $P<0.001$), as well as a significant interaction between days and sex (t=11.4, $P<0.001$), whereas no significant effect of the treatment was found. The LSD indicated that the B.W.s of males were higher than those of the females at the four time points recorded (Fig. 3B).

Discussion

The increasing use of cannabis among adolescents points out the necessity of systematic studies in appropriate animal models during the juvenile period. The present

results indicate that a chronic treatment with the cannabinoid receptor agonist CP during the peri-adolescent period affects the behavioural development of the animals, with the effects being more relevant in females. In the holeboard, which involves novelty and uncertainty (Kshama et al. 1990), the CP-treated females showed decreased EA and rearing frequency, two parameters related to general motor activity in this test (File 1992), and significantly increased %IA and head-dipping duration, whereas the frequency of head-dipping was unaltered. It has been proposed that the duration of head-dipping reflects inspective exploration, whereas the frequency of head-dipping represents inquisitive exploration (Robbins 1977). Our results support the view that these two exploratory parameters can be differentially affected by drug treatment (Viveros et al. 1996). In contrast to the results obtained in females, the males treated with CP in youth showed a significant decrease in the two exploratory parameters, whereas their general motor activity was not modified. Although in the open-field, which involves aversion (Gray 1987), the behaviour of the CP-treated animals was less modified than in the holeboard, we observed some interesting trends. Thus, the CP-treated females that were hypoactive in the holeboard showed increased immobility times in the open field. Besides, the treated animals of both sexes showed increased percentages of IA. Since rats show thigmotaxis, the increased %IA shown by the treated animals in both tests, the open field and the holeboard can be considered as an index of decreased emotionality (Gray 1987; De Cabo et al. 1995). This interpretation is supported by the results obtained in the plus-maze. In this test, the percentage of time spent in the open arms represents the best measure of anxiety (Pellow et al. 1985; File 1992), and the animals that had been treated with CP in youth showed significantly increased values for this parameter, indicative of anxiolytic-like responses. The closed-arm entries, which represent the best measure of motor activity in the plus-maze (Pellow et al. 1985; File 1992) were not modified by the pharmacological treatment. In agreement with previous studies (Gray 1987; Johnston and File 1991), the control females showed a higher motor activity and decreased levels of anxiety/emotionality than the corresponding males, in the three tests employed.

With respect to the possible neurobiological substrates underlying the effects of CP, there are several possibilities. The modifications of motor activity might be mediated by an effect of the chronic CP treatment on striatal CB1 cannabinoid receptors that mediate the acute effects of the drug on locomotion (Romero et al. 2002). In adult rats, a chronic treatment with CP, similar to that used in this study, has been shown to alter the levels of cannabinoid receptor mRNA in the striatum (Rubino et al. 1994). It is likely that these receptors are particularly sensitive to the effects of the drug during the time period of our pharmacological treatment, since within this period they are in rapid development (Rodriguez De Fonseca et al. 1993). Moreover, the sexual dimorphism found in the present study might be related to the sex differences observed in the developmental profile of the striatal CB1 receptors (Rodriguez De Fonseca et al. 1993). The present results indicate that the animals treated with CP in youth showed anxiolytic-like responses in adulthood. There is evidence indicating that endogenous enkephalins exert an anxiolytic action (König et al. 1996). Neonatal administration of THC increases the levels of methionine-enkephalin in adult rat brain (Kumar et al. 1990), and chronic administration of CP 55,940 in adult rats increases proenkephalin mRNA in several brain regions, including hypothalamic and limbic areas implicated in the regulation of anxiety (Manzanares et al. 1998). Thus, it is likely that the anxiolytic responses of our CP-treated rats in the plus-maze are mediated, at least in part, by increased levels of endogenous enkephalins. In a previous study, it was found that young male Fischer-344 rats which had received a chronic THC treatment in youth (30–50 days of age) showed an enhanced foot-shock-induced analgesia as well as a resistance to extinction of the analgesic response (Mokler et al. 1986). These data suggest that THC administered during the juvenile period may alter the endogenous opioid system. We are currently investigating whether the same CP treatment used in this study induces changes in brain cannabinoid and opioid receptors, as well as in mRNA for pro-enkephalin.

The present results show that the chronic CP treatment administered in youth did not modify the corticosterone levels of the adult animals. However, it has been shown that adult rats that had been exposed perinatally to THC showed altered corticosterone levels (Navarro et al. 1994). It is likely that there is a critical "window" of time during which the effects of chronic cannabinoid treatments produce these long-term endocrine effects. The data support previous results demonstrating a sexual dimorphism in the function of hypothalamus–pituitary–adrenal axis in rats (Viveros et al. 1988; Fernández et al. 1999).

The present results also indicate that the animals receiving the chronic CP treatment showed diminished F.I. and B.W.s during the treatment period. Our data are in agreement with previous studies showing that chronic administration of THC (Manning et al. 1971; Miczek and Dixit 1980) and acute administration of CP 55,940 (McGregor et al. 1996) inhibit F.I. and B.W. gain in rats. The effect on F.I. could be mediated by a direct effect of CP on the CB1 cannabinoid receptors located in hypothalamic centres and/or to an indirect effect resulting from the aversive state caused by the acute administration of the drug (McGregor et al. 1996; Romero et al. 2002). Besides, the reduced F.I. of the animals receiving CP could be attributable, in part, to the decreased motor activity caused by the acute administration of the drug. However, we observed development of tolerance to the effects of CP on motor activity within the period of treatment, whereas the decrease in F.I. lasted during the whole 35- to 45-day period. The development of tolerance to the motor effects of CP has been previously described in adult rats (Rubino et al. 1994). Since the animals

treated with CP recovered normal F.I. and B.W.s during the period after treatment, the long-term behavioural effects of the cannabinoid receptor agonist are not attributable to impaired nutritional state or somatic growth.

In summary, the chronic treatment with the cannabinoid receptor agonist CP 55,940 during the peri-adolescent period resulted in marked behavioural modifications in adulthood. The nature of these alterations depends on the sex of the animals and on the specific behavioural test. The present results suggest that cannabis consumption during the peri-adolescent period may induce long-term behavioural effects. It remains an open question whether these behavioural alterations might contribute to a greater proclivity to the abuse of opiates.

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