Neuropsychopharmacology (2008) 33, 806–813

www.neuropsychopharmacology.org

Augmented Acquisition of Cocaine Self-Administration and Altered Brain Glucose Metabolism in Adult Female but not Male Rats Exposed to a Cannabinoid Agonist during Adolescence

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Marijuana consumption during adolescence has been proposed to be a stepping-stone for adult cocaine addiction. However, experimental evidence for this hypothesis is missing. In this work we chronically injected male and female Wistar rats with either the cannabinoid agonist CP 55,940 (CP; 0.4 mg/kg) or its corresponding vehicle. Adult acquisition (seven 30 min daily sessions) and maintenance (fourteen 2 h daily sessions) of cocaine self-administration (1 mg/kg), food-reinforced operant learning under conditions of normal (*ad libitum* access to food), and high motivation (food-restriction schedule) were measured. Additionally, brain metabolic activity was analyzed by means of [^{18}F]-fluorodeoxyglucose positron emission tomography. During the acquisition phase, female CP-treated rats showed a higher rate of cocaine self-administration as compared to vehicle-treated females and males; no differences were found between both male groups. This effect disappeared in the maintenance phase. Moreover, no differences among groups were evident in the food-reinforced operant task, pointing to the cocaine-specific nature of the effect seen in self-administration rather than a general change in reward processing. Basal brain metabolic activity also changed in CP-treated females when compared to their vehicle-treated counterparts with no differences being found in the males; more specifically we observed a hyper activation of the frontal cortex and a hypo activation of the amygdalo-entorhinal cortex. Our results suggest that a chronic exposure to cannabinoids during adolescence alters the susceptibility to acquire cocaine self-administration, in a sex-specific fashion. This increased susceptibility could be related to the changes in brain metabolic activity induced by cannabinoids during adolescence.
Neuropsychopharmacology (2008) **33,** 806–813; doi:10.1038/sj.npp.1301467; published online 6 June 2007

Keywords: cannabinoids; PET; brain metabolism; cocaine; self-administration; gateway hypothesis

INTRODUCTION

Epidemiological studies show that the adolescent stage is a developmental period with increased risk of drug abuse (Fried *et al*, 2001; Martin *et al*, 2002; Patton *et al*, 2004; Trad, 1994). Marijuana is widely consumed at this phase of life (Gruber and Pope, 2002) and there is evidence that using the drug at this stage could facilitate later drug abuse, a phenomenon that has been termed the gateway hypothesis (Kandel *et al*, 2006). There is a great variability in human studies in regard to culture, social, and economic background, and education level of the subjects, thus it is difficult to establish causal links between adolescent marijuana consumption and adult drug abuse. Therefore, experimental studies are needed to clarify this issue (Kandel, 2003) and they are beginning to shed light on some aspects of the problem.

For instance, it has been shown that the peripubertal stage is a critical phase with special neurobehavioral plasticity and an increased vulnerability to the consequences of psychoactive drug exposure (Adriani and Laviola, 2004). Moreover, there is some evidence that a window of high vulnerability exists during adolescence, especially in the peripubertal period, where chronic cannabis administration exerts long-lasting effects on brain and behavior (Schneider and Koch, 2003; see Viveros *et al*, 2005 for review). Also, in a previous work we demonstrated that a chronic exposure to the cannabinoid agonist CP 55,940 (CP) during puberty (P35-P45) induced sex-dependent changes in locomotor activity and anxiety responses that were evident in adulthood (Biscaia *et al*, 2003). Other authors have also reported

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Received 17 November 2006; revised 2 May 2007; accepted 3 May 2007

long-term effects of cannabinoids when administered during adolescence. Schneider and Koch (2003) found impairments in sensorimotor gating, recognition memory, and performance in a progressive ratio task when the cannabinoid was administered specifically during puberty (P40-P65) but not adulthood. Schneider *et al* (2005), also observed alterations in prepulse inhibition and anxiety responses in adult rats that had been chronically treated with the cannabinoid agonist WIN 55,212-2 during puberty. Additionally, it has been reported that the cannabinoid system is still maturing during adolescence, and that the density levels (Rodriguez de Fonseca *et al*, 1993) and mRNA expression (McLaughlin *et al*, 1994) of the CB1 receptor peak between P30 and P40, and then rapidly decline to reach usual adult values (Rodriguez de Fonseca *et al*, 1993).

There have been several studies on the possible interactions between cannabinoids and the rewarding actions of psychostimulants, with no clear conclusions (see Arnold, 2005 for a review). However, most of these experiments have been performed in adult animals with their cannabinoid system already matured. It can be argued that if the stimulation of the cannabinoid system had occurred earlier in life, when it was still developing (ie during adolescence), a more profound alteration of the brain's neurochemical messengers would have been evident.

One of the systems that could be affected by a chronic cannabis exposure is the mesocorticolimbic dopaminergic system, which is widely involved in reward processing both in humans (Franken *et al*, 2005) and animals (Di Chiara *et al*, 2004). Supporting this fact, it has been shown that an adolescent exposure to cannabinoids induces long-lasting changes in the response to drugs of abuse of rat midbrain dopamine neurons (Pistis *et al*, 2004). This sensitization of the dopaminergic system could provide a mechanistic explanation for the vulnerability to cocaine addiction shown by subjects that have consumed cannabinoids during their adolescence. However, the contribution of other neurochemical systems, such as those of acetylcholine, serotonin, opioids, and glutamate among others (Adewale *et al*, 2006; Negus and Mello, 2002; Smith *et al*, 2004; Walsh and Cunningham, 1997), should not be disregarded.

In the experiments reported here, we used [^{18}F]-fluorodeoxyglucose (FDG) positron emission tomography (PET) scanning to assess the long-term neural adaptations induced by the chronic pubertal treatment. This is arguably the best approach to ascertain general neural metabolic changes and it has the advantage of having a strong translational relevance to human studies.

Most of the experimental research in the field of drug abuse has been carried out on males. However, sex-specific patterns of consumption during all phases of drug addiction are well documented (see Lynch, 2006; Roth *et al*, 2004 for reviews). So, when designing the study we considered it interesting to examine potential sex-specific effects of the cannabinoid administration.

In the present work, we chronically injected male and female rats with the cannabinoid agonist CP in the peripubertal stage (P28-P38) of adolescence (Spear, 2000) and their food and cocaine self-administration behavior in adulthood was examined. Additionally, brain glucose metabolism was analyzed by means of PET scanning using FDG.

MATERIALS AND METHODS

Animals and Drug Administration

We used Wistar albino rats of both sexes. The subjects were the offspring of rats purchased from Harlan Interfauna Ibérica SA (Barcelona, Spain), which were mated (one male $\times$ one female) in our laboratory approximately 2 weeks after their arrival. All animals were maintained at a constant temperature (20°C) and in a reverse 12-h/12-h dark/light cycle (lights on at 2000), with free access to food (commercial diet for rodents A04/A03; Panlab, Barcelona, Spain) and water unless otherwise specified. On the day of birth (postnatal day 0), litters were sex balanced and culled to 10 ± 2 pups per dam. The animals were weaned at 22 days of age.

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 28 to day 38 (11 injections), at a volume of 2 ml/kg. The dose of CP was chosen on the basis of our previous study on the long-term effects of a chronic (P35-P45) CP treatment (Biscaia *et al*, 2003). Animals were individually housed and weighted when they reached P75. All experimental procedures were carried out between 1000 and 1700. The animals used in this study were maintained in facilities according to European Union Laboratory Animal Care Rules (86/609/EEC Directive).

Apparatus

For operant food-reinforced behavior and cocaine self-administration studies, 12 operant chambers (Coulbourn Instruments, Allentown, PA, USA) were used. A lever designed to register a response when 3.0 g of force was applied was placed on the front wall of the chamber. A green stimulus light was located above the lever. Food and cocaine operant data acquisition and storage were accomplished on IBM computers (Med Associates, PA, USA).

Imaging was performed with a dedicated small animal PET scanner (piPET) (Siegel *et al*, 1999). Tomographic images were reconstructed using a 3D subset expectation maximization algorithm (3D-OSEM) (20 iterations, 5 subsets), which results in a spatial resolution of 1.65 mm full-width at half-maximum (FWHM) isotropic. The usefulness of this piPET imaging device has been previously reported (Jagoda *et al*, 2004).

Experimental Procedures

The following three experiments were performed and different sets of animals were used for each one of them.

Fixed-ratio 1 food-reinforced behavior. The experimental procedures on the first set of animals began when they were 90 days old. Before starting the training, the body weights of vehicle-exposed (VH) male ($n = 7$) and female ($n = 9$) rats and CP-exposed (CP) male ($n = 6$) and female ($n = 12$) rats were reduced to 95–90% of their free-feeding weight. This food restriction was maintained unless otherwise stated. This experiment consisted of three consecutive phases: autoshaping, fixed-ratio 1 (FR1) task with food restriction, and FR1 task with *ad libitum* access to food.

Before the actual FR1 operant task began, animals were submitted to an autoshaping food-reinforced task consisting of 30 min sessions in which food pellets (45 mg; Noyes Pellets, Lancaster, NH, USA) were either randomly delivered in intervals of 60 s average or obtained if the animal emitted an operant response (ie pressing the lever); a stimulus light was turned on right before pellet delivery signaling reward presentation. Animals emitting at least 5 operant responses were submitted to the first phase of the FR1 food-reinforced task, which consisted of 30 min sessions for 7 consecutive days throughout which the animal obtained a food pellet for each lever press. In this phase the stimulus light signaled reward availability.

After completing this phase, the animals had *ad libitum* access to food for the rest of the experiment. After recovering their original body weight, animals were submitted to the same task as in the previous phase for 5 consecutive days.

Cocaine self-administration. When the animals of the second set (males-VH, $n=11$, females-VH, $n=8$; males-CP, $n=10$, females-CP, $n=7$) reached P75 they were food restricted until they reached between 95–90% of their original body weight, and were submitted to the same autoshaping food-reinforced task described above. When the animals emitted at least five operant responses they underwent the FR1 food-reinforced task described in the previous section. When a sufficient (more than 50 responses per session) and stable (less than 10% variation across three consecutive sessions) response rate was attained, subjects were surgically prepared with an intravenous catheter placed in the jugular vein.

Put briefly, the animals were anesthetized with ketamine (40 mg/kg i.p.) and diazepam (10 mg/kg i.p.). A polyvinylchloride tubing (0.064 i.d.) was implanted in the right jugular vein approximately at the level of the atrium. The catheter was passed subcutaneously and exited in the midscapular region; it then passed through a spring tether system (Alice King, Chatham, CA, USA) that was mounted to the skull of the rat with dental cement. The animals were given at least 5 days to recover before beginning 5 extra days of FR1 food-reinforced task previous to the cocaine self-administration phase. Catheters were flushed daily with 0.5 mm of a solution of antibiotic (gentamicin, 40 mg/ml) dissolved in heparinized saline to prevent infections and to maintain catheter patency.

The drug-reinforced behavior study began when the animals reached P100, and it consisted of two phases. The first one lasted for 7 consecutive days with 30 min sessions (acquisition phase), and immediately after finishing this first part, the animals underwent 14 consecutive daily sessions of 120 min each (maintenance phase). In each session an FR1 schedule of reinforcement was followed with each lever press rendering a 1 mg/kg cocaine injection and being followed by a 30 s timeout period. A stimulus light over the lever signaled drug availability.

[^{18}F]-FDG PET scan. Basal brain glucose metabolism was measured in a third separate set of animals. To obtain brain metabolism indexes in conditions as similar as possible to those of cocaine self-administered rats, the animals of this experiment followed the same food-restriction and autoshaping protocol as described in the previous section. When they were 100 days old, the day before imaging took place, a catheter was inserted in the femoral vein under continuous isoflourane anesthesia. Female (193–214 g; CP $n=5$, VH $n=5$) and male (330–370 g; CP $n=6$, VH $n=5$) Wistar rats were then injected with [^{18}F]-FDG (1.83–1.95 mCi). After 35 min of FDG uptake, rats were imaged for 90 min and the images reconstructed by 3D-OSEM algorithm.

Data Analysis

Body weights. Body weights on the day of individualization were analyzed with a two-way analysis of variance (ANOVA).

Food-reinforced behavior and cocaine self-administration. Separate mixed three way ANOVAs were run: one for the analysis of the food-reinforced task, and another two for the acquisition and maintenance phases of cocaine self-administration. ANOVAs included two between-subjects factors (Sex (male or female) and Treatment (CP or VH)) and one within-subjects factor (Days). The dependent variable was the number of reinforcers per session. Additionally, to have more detailed information of within-session performance, separate analyses were run for the first 30, 60, and 90 min of each self-administration session during the maintenance phase.

Square roots transformations were applied when appropriate to correct the skewness in the distribution of the data and the lack of homogeneity of variances. The level of significance was set to $\alpha=0.05$. All calculations were performed with SPSS 12.0.

PET: statistical parametric mapping. To perform statistical parametric mapping (SPM) analysis, the reconstructed image sets were realigned with respect to a [^{18}F]-FDG PET template by means of a rigid registration algorithm based on the maximization of mutual information. The template image was generated by coregistering and averaging selected images from each vehicle group; a brain mask was manually segmented on the template and applied to all registered scans before performing intensity normalization. The resulting images were smoothed with a Gaussian kernel ($1.5\times1.5\times3.0$ mm FWHM), and analyzed with the SPM5 software, using an ANOVA design to detect differences between groups. Statistical significance threshold was set to uncorrected $p<0.05$ and $K=10$.

RESULTS

Body Weight

No differences due to Treatment or Treatment × Sex interaction were found in the body weight of animals in the day of individualization. As expected Sex effects were evident ($F_{1,87}=202.389$, $p<0.05$) with male rats weighing more than female ones ($Mean_{Males}=289.360$ g, $SD_{Males}=39.01$ g; $Mean_{Females}=197.464$ g, $SD_{Females}=19.45$ g).

Cocaine Self-Administration

Acquisition. The results of the repeated measures ANOVA showed a statistically significant main effect of the between-

subjects factors: Sex ($F_{1.32} = 13.630$, $p < 0.01$), Treatment ($F_{1.32} = 6.686$, $p < 0.05$), and the Sex × Treatment interaction ($F_{1.32} = 5.124$, $p < 0.05$). The analysis of the interaction revealed a significant main effect of Treatment in females ($F_{1.13} = 12.449$, $p < 0.01$), but not in males, the CP-treated females self-administering a higher number of injections per session than their vehicle-treated controls ($Mean_{Females\text{-}CP} = 4.667$, $SD = 1.45$; $Mean_{Females\text{-}VH} = 2.446$, $SD = 1.15$) (see Figure 1a). No effects of the within-subject factor Days were found indicating a stable acquisition in all groups.

Maintenance. Figure 1b shows the mean number of injections per session during the maintenance phase. No effects of the between-subjects factors Sex, Treatment, or the Sex × Treatment interaction were found in any of the time frames analyzed (30, 60, 90, and 120 min). A significant effect of the within-subjects factor Days was found ($F_{13.416} = 2.373$, $p < 0.05$) indicating a progressive increase in performance, presumably due to the longer length of sessions in this phase.

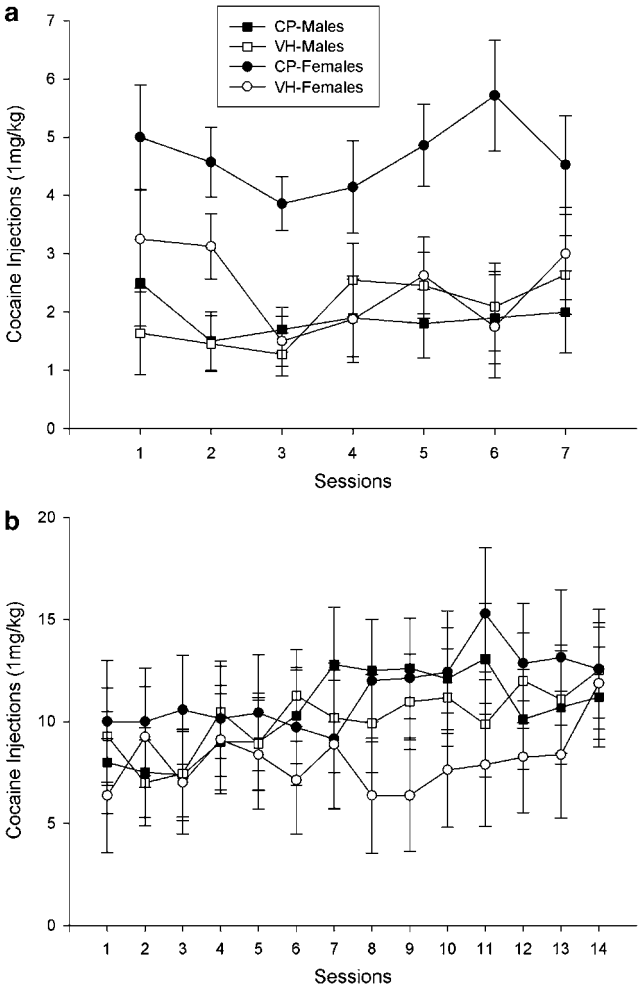

Figure 1 (a) Acquisition of cocaine self-administration: mean number of injections per session across the seven daily acquisition sessions (30 min duration). As observed female CP-treated rats (CP-Females) self-administered a higher number of injections per session when compared to the rest of the groups. No other significant differences were found. The lack of effect of the Day factor indicates a stable self- administration in all groups. (b) Maintenance of cocaine self-administration: performance during the maintenance phase of cocaine self-administration (14 daily sessions of 2 h duration). No differences among groups were found. However the effect of Days was significant, indicating a progressive increase in the responses across days, presumably due to the change in the session's duration.

Food-Reinforced Behavior

To test the specificity of the effects observed in cocaine self-administration, we ran a parallel experiment with food pellets as reinforcers instead of cocaine infusions. In this case, as seen in Figure 2, no effect of either the Sex, the Treatment, or the Sex × Treatment interaction was evident neither during the food-restriction phase nor during the *ad libitum* phase.

[^{18}F]-FDG PET Scan

The SPM technique showed a significant hypoactivation of the amygdalo-enthorinal area ($t = 1.86$, $p < 0.05$; $K = 10$) and a hyperactivation of the frontal cortex ($t = 1.86$, $p < 0.05$; $K = 80$) in the CP-treated females as compared to VH-injected females. No significant differences were found in males (see Figure 3).

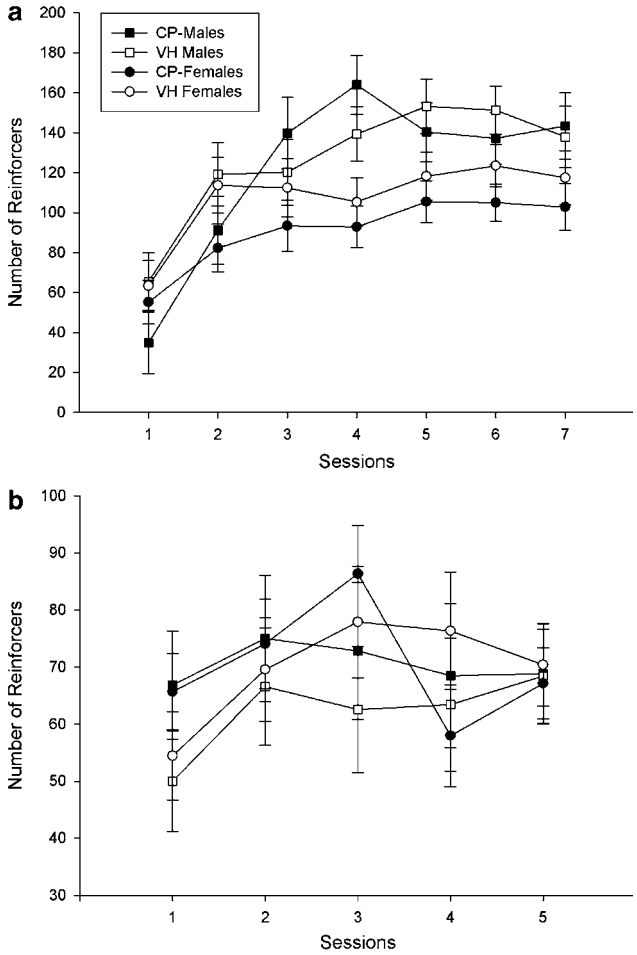

Figure 2 FR1 food-reinforced operant behavior: food-reinforced behavior during food restriction (a) and *ad libitum* (b) conditions. The number of reinforcers earned per session is shown. No differences were observed in any of the conditions among the groups.

DISCUSSION

In this work, we have evaluated the long-term effects of a chronic treatment with the cannabinoid agonist CP during adolescence (P28-P38) on adult acquisition and maintenance of cocaine self-administration, food-reinforced operant learning, and brain metabolic activity. During acquisition, the group of female rats exposed to CP during puberty exhibited higher adult cocaine self-administration rates when compared to the other groups. This effect was not present during maintenance and no other differences were observed. Additionally, no differences were evident in the operant food-reinforced task, indicating that the effect on self-administration rates was specific to cocaine and not a general change in reward processing. Moreover, the chronic treatment with CP exerted changes in brain glucose metabolism in CP-treated females as compared to their vehicle counterparts. These alterations consisted of a hyperactivation of the frontal cortex and a hypoactivation of the amygdalo-enthorinal area. No changes were observed in males.

As stated in the 'Introduction', until very recently the gateway hypothesis lacked robust experimental validation. A few studies had examined the causal link between a prior tetrahydrocannabinol (THC) exposure and a subsequent enhanced drug self-administration; however, they used adult THC pre-treated rats that subsequently underwent heroin or cocaine self-administration (Panlilio *et al*, 2007; Solinas *et al*, 2004). Since these designs did not incorporate the developmental component that is cardinal to the gateway hypothesis, a full experimental validation of the hypothesis was still missing. To our knowledge, the first time the gateway hypothesis has been confirmed experimentally is that of Ellgren *et al* (2007) who have reported higher heroin self-administration rates during adulthood in male rats treated with THC during adolescence.

In the context of psychostimulants, Ellgren *et al* (2004) examined at different periods the effects of amphetamine on dopamine levels and behavior following cannabinoid exposure during adolescence. No support for the hypothesis was found since no changes were observed either in adolescence or adulthood in dopamine or locomotor responses to amphetamine.

Although cannabinoids and dopamine have been shown to interact at several levels (Andersson *et al*, 2005; Gardner, 2005; Hermann *et al*, 2002), the relationship between cannabinoids and the effects of psychostimulant drugs is currently a matter of debate (see Arnold, 2005 for a review). For example, Fattore *et al* (1999) showed that an acute administration of the cannabinoid agonist WIN 55,512–2 (WIN) decreased cocaine self-administration, and Soria *et al* (2005) reported that mice lacking the CB1 receptors exhibited reduced reinforcing efficacy of cocaine. In contrast to this, Tanda *et al* (2000) found that the administration of the CB1 receptor antagonist rimonabant (SR141716A) did not affect cocaine self-administration rates in squirrel monkeys. Additionally, normal behavior in cocaine self-administration and conditioned place preference paradigms has been found in CB1 knockout mice (Cossu *et al*, 2001; Martin *et al*, 2000).

Cocaine is known for exerting both rewarding and anxiogenic effects (Ettenberg, 2004), so the higher self-administration rates shown by CP-females in our study could reflect either an increase in the rewarding actions of cocaine or a decrement in its anxiogenic effect (Panlilio *et al*, 2007). There is evidence that fails to support the former speculation. First, the chronic CP treatment does not seem to affect the rewarding values of natural reinforcers (since no effects were observed in the food-reinforced task), neither in conditions of high motivation (food deprivation) nor in normal basal conditions (*ad libitum* phase). Second, Ellgren *et al* (2004) did not find changes in dopamine release in response to amphetamines after a THC treatment during adolescence, providing further evidence against a change in the rewarding/hedonic value of the drug *per se*.

A change in anxiety responses in CP-treated animals has been previously reported by our group (Biscaia *et al*, 2003). In this study, using the same doses and number of CP injections, we observed that both female and CP-treated animals during early adolescence (P35–45) showed diminished anxiety responses in the elevated plus maze. Although these effects were additive and not interactive, it can be

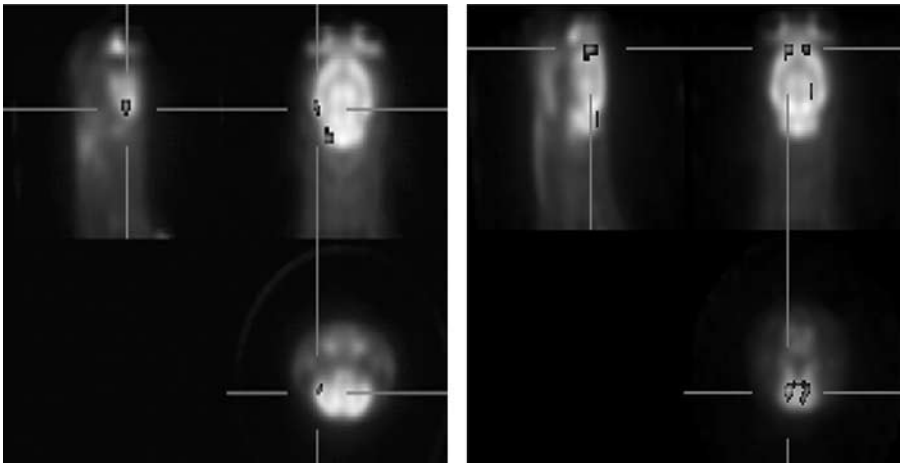

Figure 3 Brain glucose metabolism as measured by PET imaging: SPM revealed significant differences in female rats exposed to the cannabinoid agonist during adolescence. Lower metabolism in amygdalo-entorhinal area (left image) and higher metabolism in frontal cortex (right image) of CP-Females as compared to their vehicle-treated controls.

suggested that a higher reduction in anxiety occurred in CP female rats. This can be hypothesized to be a putative mechanism explaining our results in the acquisition of cocaine self-administration. Presumably, CP females might experience cocaine as less anxiogenic and therefore they might self-administer more injections of the drug. This hypothesis needs further experimental testing and CP should be administered in the same age interval used in the present study (P28-P38).

An alternative explanation would point to changes in general locomotor activity that could have been induced by the treatment in the females; these motor alterations could result in an elevated frequency of exploratory behavior (and therefore higher chances of pressing the lever during the acquisition phase). In this sense, it has been shown that female rats perinatally exposed to THC showed persistent changes in spontaneous locomotor activity (Navarro *et al*, 1994; Rubio *et al*, 1995). Additionally adult rats treated with the CB1 agonist WIN exhibited an increased locomotor activity as measured in the open field, but only at the lowest dose tested (0.6 mg/kg) (Drews *et al*, 2005). Although appealing, this hypothesis is not supported by more specific experimental work since we have found a decrement rather than an increment in locomotor activity in CP-treated females using a very similar design to the one presented here (Biscaia *et al*, 2003). Moreover, Schneider *et al* (2005) did not find any change in this respect in adult rats treated with WIN during adolescence.

Surprisingly, we have not observed the typical pattern of sex differences in self-administration (Lynch, 2006) in the VH groups (ie females acquiring cocaine self-administration faster and self-administering higher amounts of the drug), a fact which might be due, at least in part, to the particular self-administration schedule used here, and the dose of cocaine employed. In this sense, it has been recently reported that sex-differences in cocaine self-administration disappear when low FR ratios are used and when cocaine doses are lower than 3 mg/kg (Kantak *et al*, 2007). Interestingly, the treatment seemed to boost this latent sex-specific effect in the acquisition phase.

The chronic treatment employed in this study also induced changes in brain glucose metabolism. Although the precise onset of these changes was not evaluated, they were still evident at the age when self-administration began. Our data point to a hyperactivation of the frontal cortex and a hypoactivation of the amygdalo-enthorinal area caused by the chronic treatment with CP during early adolescence. In addition, these changes only occurred in CP-treated females, a fact which parallels our behavioral findings.

The results of the present study differ from those reported by other groups that have employed whole brain imaging techniques such as 2-DG autoradiography to measure brain glucose metabolism. In that kind of studies, a general reduction in glucose utilization after THC administration has been consistently reported (Freedland *et al*, 2003; Whitlow *et al*, 2002, 2003). However, in those experiments, the cannabinoid was always administered in the adult stage, and on the other hand, the drug used was THC and not CP. Interestingly, human studies have shown an increased glucose metabolism in the frontal cortex (Volkow *et al*, 1991), especially in the right hemisphere after an intravenous THC injection. The increased activity found in the frontal cortex could be derived from the persistent stimulation of the dopaminergic mesocorticolimbic pathway caused by the chronic treatment. Thus, the activation of the cannabinoid receptors has been shown to increase the activity of meso-prefrontal activity of dopaminergic neurons (Diana *et al*, 1998). Perhaps, a repetitive stimulation of the receptor in a particularly plastic stage might have increased the activity of the frontal cortex in a persistent fashion. Regarding this possibility, only one report in the literature has explored the importance of the age of onset of cannabinoid consumption, and it has been carried out in human subjects (Wilson *et al*, 2000). The authors found that initiating marijuana consumption during adolescence seems to lead to greater brain cerebral flow alterations induced by THC. At the behavioral level the hyperactivation of the frontal cortex seen in CP-treated females could partially explain (among other behavioral disruptions) their heightened vulnerability to psychostimulants, due to the importance of this area in the circuitry of drug addiction (Tzschentke and Schmidt, 2000).

Another interesting finding in the present work is the hypoactivation seen in the amygdalo-enthorinal area of CP-treated females as compared to the other groups. This area is believed to play a role in the storage of long-term memories before their entry into the perirhinal cortex where they are thought to be kept for longer periods of time until recovery (Izquierdo and Medina, 1997). Therefore, a hypoactivation in this area might indicate some deficits in long-term memory storage, at least in the first stages of its initial consolidation. Sim *et al* (1996) reported decreases in WIN-stimulated GTPγ^{35}S binding in the entorhinal cortex after a chronic THC treatment. This desensitization of CB1 receptors might account for the hypoactivation observed in this brain region.

In conclusion, we have shown that a chronic treatment with a cannabinoid agonist during a specific stage of adolescence is able to induce behavioral and neural changes that are still evident in adulthood and that could be mediating the enhanced vulnerability to cocaine self-administration seen in female rats pubertally treated with the cannabinoid agonist CP. The sex-specificity of the effects needs to be further explored and the role of estrogens as putative mediators of our findings is currently the focus of ongoing experiments.

ACKNOWLEDGEMENTS

Cocaine chlorhydrate was kindly provided by the Dirección General de Estupefacientes (Spain). We thank Miguel Biscaia, Gonzalo Higuera, and Paloma Díaz for critical comments on a previous version of this manuscript and Luis Troca, Rosa Ferrado, and Alberto Marcos for technical assistance. This work was supported by Grants FIS G03/05 (Red de Trastornos Adictivos), BSO2001-1099, FIS 01-05-01, Plan Nacional sobre Drogas (PNSD) 2001–2003, PNSD 2004–2007, GR-SAL/0260/2004 to EA and Grants INT/2012/2002, CB06/01/0079, and CENIT (2006–2009) to MD.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare that except for income received from their primary employer, no financial support or compensa-

tion has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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