
BRIEF ARTICLE

Chronic Cannabinoid Administration to Periadolescent Rats Modulates the Metabolic Response to Acute Cocaine in the Adult Brain

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Abstract

Purpose: To analyze brain metabolic response to acute cocaine in male and female Wistar rats with or without a history of cannabinoid exposure during periadolescence.

Procedures: The synthetic cannabinoid agonist CP 55,940 (CP) or its vehicle (VH), were administered to male and female rats during periadolescence. When these animals reached adulthood, saline and cocaine-induced changes in 2-deoxy-2-[¹⁸F]fluoro-D-glucose (FDG) uptake were studied by positron emission tomography.

Results: The baseline (post-saline) metabolism in the septal nuclei was higher in CP-females than in VH-females, although septal metabolism was lower in CP-females after cocaine, reaching similar values to those of VH-females at baseline. Cocaine did not affect metabolism in VH-females. Periadolescent cannabinoid treatment did not influence baseline metabolism in males although cocaine reduced the FDG uptake in the dorsal striatum of males that received the VH but not CP.

Conclusions: These results suggest that cannabinoids during periadolescence modify baseline and cocaine-evoked brain metabolism in a sex-dependent manner. In the case of CP-females, the involvement of septal metabolic alterations in their susceptibility to the rewarding effects of cocaine should be further investigated.

Key words: Cannabinoids, Cocaine, Dorsal striatum, PET, Septal nuclei

Significance: In the present report, we present combined PET/MR imaging evidence suggesting that periadolescent cannabinoid treatment to rats modifies the metabolic response to cocaine in adulthood in a sex-dependent manner. We also show for the first time in the rat that cocaine is associated with decreased metabolism in the dorsal striatum. This work extends previous findings by showing how the brain metabolic changes induced by cocaine are modulated by a chronic cannabinoid treatment.

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Introduction

There is significant evidence that during adolescence, marijuana is the most widely consumed illicit drug [1]. It has been hypothesized that its consumption at this stage of life might predispose individuals for later cocaine abuse [2, 3]. Although the number of animal studies that support this association is limited, we recently showed that chronic

cannabinoid treatment during periadolescence enhances the acquisition of cocaine self-administration behavior in adult female but not male Wistar rats [4]. In order to understand and further characterize this effect, it would be useful to determine how cocaine acts on the brain in animals exposed to cannabinoids.

Among the metabolic changes induced in the rat brain by acute cocaine administration is the enhanced local glucose metabolism in dorsal striatum, globus pallidus, substantia nigra pars reticulata, subthalamic nucleus, and cerebellar vermis, as well as a reduction in its metabolism in the habenulla [5]. There are also studies showing that acute cocaine administration decreases global brain metabolism in humans, with specific decreases in neocortical areas, the basal ganglia, and regions of the hippocampal formation, thalamus, and midbrain [6]. Indeed, a long-lasting decrease in cortical metabolism (still evident even after 3–4 months of a drug-free period) has also been observed in cocaine abusers [7].

Positron emission tomography (PET) constitutes an invaluable tool to investigate changes in brain function as a result of pharmacological manipulations. The non-invasive nature of the technique and its applicability to humans makes it an excellent tool to study neuropsychopharmacological issues in animals [8]. Here, we have used combined PET/Magnetic Resonance (MR) imaging to examine the brain metabolic response to acute cocaine administration in adult male and female rats that had been chronically treated with a cannabinoid agonist during periadolescence. The results obtained could provide further insights into the neural alterations that mediate the enhanced cocaine self-administration behavior in rats with a history of early cannabinoid exposure [4].

Materials and Methods

Animals, Drug Administration, and Experimental Protocol

We used male and female Wistar albino rats bred in our laboratory from rats purchased from Harlan Interfauna Ibérica S.A. (Barcelona, Spain) that were mated approximately 2 weeks after their arrival (one male \times one female). All animals were maintained at a constant temperature (20°C) and on a reverse 12-h/12-h dark/light cycle (lights on at 2000 hours), with free access to food and water. On the day of birth (postnatal day—P0), the litters were gender-balanced and culled to 10 ± 2 pups per dam. Rats were weaned P22 and housed in groups of 3–4 animals of the same sex per cage. Every attempt was made to minimize pain and discomfort of the experimental animals. The rats used in this study were maintained in facilities that complied with European Union Laboratory Animal Care Rules (86/609/EEC Directive) and the Guide for the Care and Use of Laboratory Animals (National Institutes of Health). All the procedures performed were carried out in accordance with the same guidelines and were approved by the Ethics Board of the Universidad Nacional de Educación a Distancia.

CP 55,940 (CP, Tocris; 0.4 mg/kg) or its corresponding vehicle (VH, ethanol:cremophor:saline [1:1:18]; cremophor: Fluka Bio-ChemiKa) were administered once daily via the intraperitoneal

(i.p.) route, from P28 to P38 (11 injections), at a volume of 2 mL/kg. Accordingly, four groups of animals were established: VH-male ($n=7$), VH-female ($n=9$), CP-male ($n=8$) and CP-female ($n=9$). The dose and duration of the CP treatment were chosen on the basis of previous studies on the long-term effects of chronic periadolescent CP treatment [4, 9, 10]. Animals were housed individually when they reached P75 in order to leave sufficient time between individual housing and the metabolic studies to avoid any non-specific effects of isolation stress on the metabolic measurements. An intravenous catheter was implanted when the rats reached approximately P93. Surgical procedures were performed under ketamine (40 mg/kg intraperitoneal) and diazepam (10 mg/kg i.p.) anesthesia. The catheter consisted of a polyvinylchloride tubing (0.064 i.d.) that was implanted into the right jugular vein at approximately the level of the atrium, and that passed subcutaneously to exit the midscapular region. Catheters were flushed daily with 0.5 mL of an antibiotic solution (Gentamicin 40 mg/mL) dissolved in heparinized saline in order to prevent infection and to maintain patency. All experimental procedures were carried out between 1000 hours and 1700 hours.

FDG-PET

PET imaging was carried out in two separate sessions: the first session following saline administration (P100) and the second session following cocaine administration (P102). On P100, the animals received an acute intravenous (i.v.) injection of saline (1 mL/kg of 0.9% NaCl: "baseline metabolism") and on day P102, the same animal received an acute i.v. injection of cocaine (1 mg/kg) through the jugular catheter.

2-deoxy-2- ^{18}F fluoro-D-glucose (FDG: 1.99 ± 0.24 mCi) was injected through the catheter 10 min after the saline or cocaine. FDG uptake occurred over 35 min and at the end of the uptake period, the animals were anesthetized with isoflurane (5% for induction and 1–1.5% for maintenance in 100% O₂). Subsequently, the rats were imaged for 60 min in a dedicated small-animal PET scanner (rPET, SUINSA Medical Systems, Madrid) [11].

Tomographic images were reconstructed with a 3D filtered back projection (3D-FBP) algorithm [12] using a twelfth-order Butterworth filter at 35% Nyquist frequency cutoff. The transaxial and axial resolutions of the PET scanner were 1.65 mm and 1.9-mm full width at half-maximum (FWHM), respectively. The voxel size of the reconstructed images was isometric (0.81 mm³), the energy window was 400–700 keV, and decay, alignment, normalization, and deadtime corrections were all applied.

All images were spatially registered using rigid transformations with an automatic algorithm that optimized normalized mutual information [13], thereby enabling voxel-to-voxel comparisons. Since the sizes of the male and female rat brains were slightly different, images were registered separately. Spatially registered images were averaged in each group and two templates were generated (male and female). All PET data were smoothed with a 2-mm FWHM isotropic Gaussian kernel using the Statistical Parametric Mapping software package (SPM5, version 1782, Wellcome Department of Cognitive Neurology, London, UK). To ensure that only voxels mapping brain tissue were included in the analysis, two brain masks (male and female) were manually segmented on the templates and applied to their corresponding PET studies to remove extracerebral voxels. Image intensity was normalized to the same overall brain average value (100%).

Magnetic Resonance Study

One male and one female adult Wistar albino rats (approximately P100) was anesthetized with sevoflurane (1%) and monitored. After global shimming, a rapid acquisition with relaxation enhancement (RARE) sequence was acquired (TR, 4,062 ms; TE, 33 ms; flip angle, 90°; RARE factor, 8; FOV, 3.7×3.7 cm; slice thickness, 0.8 mm; matrix, 256×256; number of slices, 37; number of accumulations, five; scan time, 11 min) in a Bruker Biospec 70/20 USR scanner (7 T) using a rat head surface array coil centered on the brain. Non-uniform intensity caused by the surface coil was corrected with an automatic algorithm (*n3*: [14]) applying the following parameters: distance 8 mm, 250 iterations, FWHM 0.15 mm, stop criteria 0.0001. A brain mask created by region-growing automatic segmentation was used to avoid including the background noise in the correction.

PET templates for males and females were mapped semi-automatically to their corresponding MR images and the rigid transformations obtained were applied to statistical parametric maps. As the anatomical resolution of PET is low, the fusion between MR images and statistical parametric maps was used to aid the anatomical localization of statistically significant differences.

Data Analysis

Male and female rats were analyzed separately by means of a mixed ANOVA (carried out with SPM5, which follows a voxel-based approach to data analysis), with periadolescent exposure (CP, VH) as the between-subjects factor and adult administration (saline, cocaine) as the within-subjects factor. The design matrix also modeled subjects and interactions between both factors. A threshold of $p < 0.001$ (uncorrected) was used to determine the significant activation for each voxel. To reduce type I error, a 10-voxel clustering (spatial-extent) threshold was also applied and hence, significant regions smaller than ten contiguous activated voxels were not admitted.

Results

In male rats, the SPM analysis showed no differences in baseline (saline-associated) metabolism following periadolescent treatment. However, after an acute cocaine challenge, a significant lower metabolism was observed in the dorsal striatum of VH-males, while no changes were detected in male rats that had received CP in periadolescence (Table 1; Fig. 1, Panel 1; Fig. 2).

In female rats, those pre-treated with CP exhibited a higher baseline metabolism than VH-treated controls, mainly in regions comprising septal nuclei. Interestingly, these differences disappeared after acute cocaine challenge, as a result of the decreased septal metabolism observed in CP-females

(Table 1; Fig. 1, Panel 2; Fig. 2). Control females showed no alteration in FDG uptake after cocaine administration.

Discussion

In this study, we have used PET/MR imaging combined with a SPM analysis to explore the metabolic responses in the brain to acute cocaine administration in male and female rats with a history of chronic periadolescent exposure to a cannabinoid agonist.

When imaged as adults, the baseline metabolism in the septal nuclei of CP-females is higher than in VH-females. This difference disappears after cocaine administration, due to a reduced metabolic response to cocaine in CP-exposed female rats. Interestingly, adult VH-females show no metabolic response to cocaine administration. There are no baseline differences in the dorsal striatum between male rats exposed to CP or VH, but VH-males also experience a metabolic decrease after receiving cocaine. CP-exposed males do not exhibit this decrease in FDG uptake.

Septal Nuclei

The higher baseline metabolism in the septal nuclei of CP-females is of special interest due to the fact that septal lesions have been shown to increase the sensitivity to cocaine place-preference conditioning in rats [15]. Accordingly, it could be argued that the higher baseline metabolism in the septal nuclei of CP-females could promote hyposensitivity to the acute rewarding effects of cocaine. This hyposensitivity of CP-females may in turn be related to their higher rates of cocaine self-administration during acquisition [4]. However, other functional implications of this altered septal metabolism, such as changes in emotional behavior, should also be considered. As regards this, chronic cannabinoid exposure during adolescence induces a depressive phenotype in adult female rats [16]. Accordingly, septal nuclei metabolism alterations have been observed in PET imaging studies in rats subjected to chronic mild stress, an animal model of depression [17].

Dorsal Striatum

No difference is observed in dorsal striatal baseline metabolism in males and cocaine only decreases metabolism in VH-males, as CP-males do not seem to be affected by the challenge. Although CP- and VH-males do not differ in their

Table 1. Rat brain regions showing statistically significant differences ($p < 0.001$ uncorrected, $k=10$)

Variables	Regions	Z score (max.)	Cluster size ^a
Males	VH (saline)>VH (cocaine)	Dorsal striatum (right+left)	75
	VH (cocaine)<CP (cocaine)	Dorsal striatum (right+left)	101
Females	CP (saline)>CP (cocaine)	Septal nuclei	79
	CP (saline)>VH (saline)	Septal nuclei	23

^aNumber of significant voxels in the region associated to the cluster

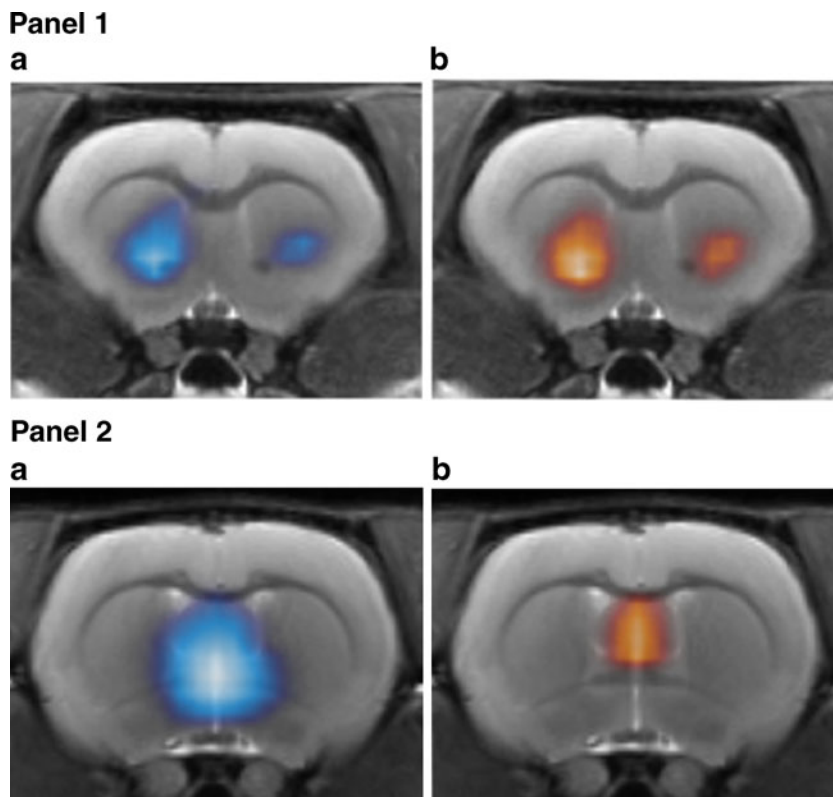


Fig. 1. Panel 1: Representative coronal images of VH-males showing hypoactivation (*blue*) in the dorsal striatum after cocaine administration when compared to the effects of saline administration (**a**), and hyperactivation (*orange*) in CP-males of dorsal striatum post-cocaine metabolism when compared with the post-cocaine metabolism of VH-males (**b**). *Brighter and darker colors* indicate larger and smaller significant differences, respectively. **Panel 2:** Representative coronal images of CP-females showing hypoactivation (*blue*) in the septal nuclei after cocaine administration when compared to the effects of saline administration (**a**), and hyperactivation (*orange*) in CP-females of septal baseline metabolism (saline) when compared with the baseline metabolism of VH-females (**b**). *Brighter and darker colors* indicate larger and smaller statistically significant differences, respectively.

cocaine self-administration behavior [4], the cocaine-induced decrease in striatal metabolism in VH-males and the lack of effect of cocaine in CP-males could have some impact on other behavioral indexes (such as cocaine-induced locomotor activity).

To our knowledge, this is the first work that reports that acute cocaine is associated with lower brain metabolism in the dorsal striatum of the rat. Indeed, the decrease in FDG metabolism observed in the dorsal striatum of VH-males after acute cocaine administration resembles its effect on brain metabolism in mice [18], monkeys [19] and human beings [6], although it is in opposite direction to the results previously obtained in one study performed in rats where cocaine was shown to increase glucose metabolism in the dorsal striatum [5]. In another rat study, i.v. cocaine did not alter local glucose utilization in this structure [18]. The reason for these discrepancies remains unclear, although several explanations can be suggested: in the first study [5], cocaine was injected by the i.p. route at a dose of 30 mg/kg, implying different cocaine bioavailability and pharmacokinetics from those observed after i.v. injection. In the second study [18], the same dose of cocaine and the same route of administration as the ones reported here were used, the

divergences may then be related to the different strains of rats used (Spague Dawley vs. Wistar) or the inherent differences between the autoradiography and PET techniques. In this sense, while absolute brain metabolism was previously measured using post-mortem autoradiography of

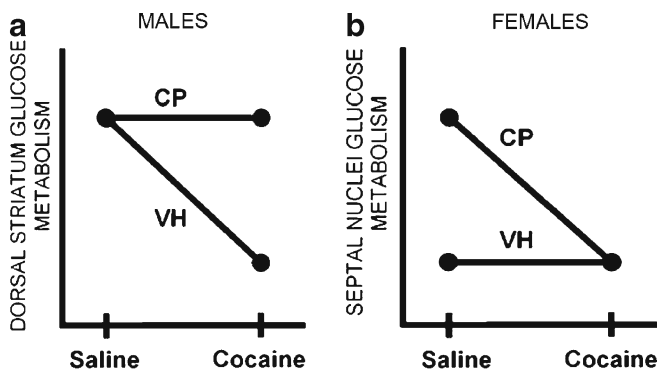


Fig. 2. Interaction plots showing relative metabolism for the dorsal striatum of males (**a**) and the septal nuclei for females (**b**). Interactive effects between chronic periadolescent cannabinoid/vehicle exposure and adult cocaine or saline administration are shown. Values are expressed as mean \pm SEM and referred to total brain metabolism (100%).

2-deoxy-D-glucose [18], we used FDG-PET scanning of the live animal under isoflurane anesthesia. Moreover, in this earlier study [18] a set of calibrated standards was used to normalize tissue activity, whereas our data were normalized against total brain metabolism. These differences may explain the divergent results obtained in the present work and those previously published [5, 18].

The underlying neural mechanisms mediating the effects described in this work still remain unclear; however, long-term alterations in the dopamine transporter or receptors do not seem to be involved [20]. Other factors such as changes in cocaine pharmacokinetics deriving from the cannabinoid treatment could also explain our results. Additionally, the sex-dependent nature of the effects reported here could be attributed to long-term changes in hormone levels, such as estradiol or corticosterone, both of which are affected by cannabinoids [21–23]. However, when we examined this possibility, we found no alterations in corticosterone or estradiol in animals exposed to CP [10].

In conclusion, this is the first report to show using FDG-PET that acute cocaine administration has different effects on brain metabolism in function of prior periadolescent cannabinoid exposure. Additionally, this long-term effect of cannabinoids depends on the sex of the animals. Further studies must now evaluate the functional implications of the altered septal and striatal metabolism reported here in cocaine's stimulant and rewarding actions.

Acknowledgements. The authors thank Alberto Marcos, Rosa Ferrado, and Luis Troca for their technical assistance and Dr. Mark Sefton for editorial assistance. The authors declare that they are entirely responsible for the scientific content of this paper. This work was supported by grants from the Ministerio de Educación y Ciencia (Grants n° SAF2004-08148 and SAF2007-064890); Ministerio de Sanidad y Consumo (Grants RD06/00170029 of Instituto de Salud Carlos III, PNSD 2004–2007 and 2008–2010); Dirección General de Investigación de la Comunidad de Madrid (Grant S-SAL/0261/2006, I+D CANNAB-CM Consortium); and UNED (Plan de Promoción de la Investigación) to EA, and grants from the “Ministerio de Ciencia y Tecnología” (TEC2004-07052-C02-01/TCM), “Ministerio de Sanidad y Consumo” (CIBER CB06/01/0079, PNSD 2007–2010, FIS CP08/00017), “Ministerio de Industria” (CENIT program) and “Fundación de Investigación Médica Mutua Madrileña” (2007–2010 and 2008–2011) to MD.

Conflict of Interest. The authors declare that they have no conflict of interest.

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