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Effects of perinatal exposure to Δ^9-tetrahydrocannabinol on operant morphine-reinforced behavior

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Abstract

The present study examined the effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) when administered during the perinatal period on morphine self-administration in adulthood. To this end, pregnant Wistar rats were daily exposed to Δ^9-THC from the fifth day of gestation up to pup weaning, when they were separated by gender and left to mature to be used for analyses of operant food- and morphine-reinforced behavior in a progressive ratio (PR) schedule. We also analyzed dopaminergic activity (DOPAC/DA) in reward-related structures during specific phases of the behavioral study. In both reinforcement paradigms, food and morphine, females always reached higher patterns of self-administration than males, but this occurred for the two treatment groups, Δ^9-THC or vehicle. These higher patterns measured in females corresponded with a higher DOPAC/DA in the nucleus accumbens prior to the onset of morphine self-administration in comparison to males. Interestingly, DOPAC/DA was lower in Δ^9-THC-exposed females compared to oil-exposed females and similar to oil- and Δ^9-THC-exposed males. In addition, Δ^9-THC-exposed females also exhibited a reduction in DOPAC/DA in the ventral tegmental area, which did not exist in males. All these changes, however, disappeared after 15 days of morphine self-administration and they did not reappear after 15 additional days of extinction of this response. Our data suggest that females are more vulnerable than males in a PR schedule for operant food and morphine self-administration; perinatal Δ^9-THC exposure is not a factor influencing this vulnerability. The neurochemical analysis revealed that the activity of limbic dopaminergic neurons prior to morphine self-administration was higher in females than males, as well as that the perinatal Δ^9-THC treatment reduced the activity of these neurons only in females, although this had no influence on morphine vulnerability in these animals.

Keywords: Perinatal Δ^9-THC exposure; Cannabinoids; Morphine self-administration; Dopamine; Opioid-related processes

1. Introduction

Cannabis derivatives are the most commonly used illicit drug in Western societies, particularly among young people (for a recent review, see Ashton, 2001), being consumed even by women during pregnancy and lactation (for a recent review, see Fried and Smith, 2001). The consumption of this drug during the perinatal period has been reported to result in a variety of disturbances in the development of their offspring, because, like other psychoactive drugs, cannabinoids, the psychoactive ingredients of marijuana, can cross the blood–brain and placental barriers and be secreted in the maternal milk (for review, see Dalterio, 1986; Fernández-Ruiz et al., 1992; Behnke and Eyler, 1993). Through this way, cannabinoids affect the ontogeny of various neurotransmitter systems (Walters and Carr, 1986, 1988; Rodríguez de Fonseca et al., 1991; Fernández-Ruiz et al., 1992; Bonnin et al., 1994, 1995; Molina-Holgado et al., 1996; García-Gil et al., 1996, 1999; for review, see Fernández-Ruiz et al., 1999, 2000) leading to changes in different behavioral patterns (Dalterio, 1986; Mokler et al., 1987; Murphy et al., 1990; Fernández-Ruiz et al., 1992; Navarro et al., 1994, 1996; for review, see Fernández-Ruiz et al., 1999, 2000).

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Endogenous opioids are among the neurotransmitters affected by perinatal cannabinoid exposure (Kumar et al., 1990; Manzanares et al., 1999; Pérez-Rosado et al., 2000, 2002), which, when animals mature, produce changes in the sensitivity to morphine in relation to its analgesic effects (Vela et al., 1995) or its reinforcing properties (Vela et al., 1998). In the study by Vela et al. (1998), it was observed that adult female rats born from mothers treated with Δ^9-tetrahydrocannabinol (Δ^9-THC) during gestation and lactation exhibited a significant increase in the acquisition rate of morphine self-administration behavior compared to females born from vehicle-exposed mothers, as well as compared to both oil- and Δ^9-THC-exposed male offspring, but there were not any significant differences on acquisition of operant food-reinforced behavior. These behavioral changes were related to increased density of μ-opioid receptor binding sites (Vela et al., 1998) and decreased mRNA levels for proenkephalin (Corchero et al., 1998) in various limbic and motor regions of Δ^9-THC-exposed females, events that are associated with increased vulnerability to morphine (Ambrosio et al., 1999; Martín et al., 1999).

Vela et al. (1998) used a low fixed-ratio schedule of reinforcement to reveal the vulnerability to morphine of Δ^9-THC-exposed females. These low-value ratio schedules are useful for determining whether a particular drug is reinforcing, but they, however, provide limited information about the relative magnitude of the reinforcing efficacy of different drugs (Griffiths et al., 1979; Arnold and Roberts, 1997). So, in the present study, we wanted to repeat this previous study (Vela et al., 1998), but introducing two new parameters: (i) we used a progressive ratio (PR) schedule (Hodos, 1961) to determine the magnitude of the reinforcing efficacy of morphine in adult rats perinatally exposed to Δ^9-THC (at the same dose used in Vela et al., 1998) and (ii) we also analyzed the activity of mesolimbic dopaminergic neurons, which are the major neurobiological substrate activated by habit-forming drugs (for review, see Spanagel and Weiss, 1999). With this PR schedule, the number of lever presses required to obtain a drug injection is increased with successive injections, which presumably reflects better the motivation of the animal to self-administer the drug (Mello et al., 1988; Depoortere et al., 1993; Cohen and Sanger, 1994). In addition, to clarify whether the potential effects of perinatal exposure to Δ^9-THC on morphine self-administration are due to a general effect of this cannabinoid on any potential reinforcers or whether they are specific for morphine, we also examined the acquisition of operant food-reinforced behavior in a separate group of Δ^9-THC-exposed animals.

2. Methods

2.1. Animals, treatment, and sampling

Female virgin Wistar rats were housed from birth in a room with a controlled photoperiod (08:00–20:00 h light) and temperature (23 ± 1 °C) and with free access to standard food (Panlab, Barcelona, Spain) and water. At adult age (>8 weeks of life; 200–250 g), daily vaginal smears were taken between 10:00 and 12:00h, and only those animals exhibiting three or more consistent 4-day cycles were used in this study. Females in the proestrous phase were allowed to stay with a male for mating and a new vaginal smear was taken on the next day. Those animals showing the presence of sperm cells were accepted as probably pregnant and used for the cannabinoid exposure studies. The day on which sperm plugs were found was designated the first day of gestation. Experimental procedures and care of animals conformed to directives of the European Union on Laboratory Animal Care (directive 86/609/EEC). Δ^9-THC, kindly supplied by the National Institute on Drug Abuse (Baltimore, MD), was prepared in a sesame oil solution. Pregnant females received a daily oral dose of Δ^9-THC (5 mg/kg weight) from the fifth day of gestation according to our previously published model (Fernández-Ruiz et al., 1992; Bonnin et al., 1995). This dose was chosen because of two reasons: (i) it was the same dose as used in the previous experiments of morphine self-administration that serve as comparative study (Vela et al., 1998) and (ii) it is an active dose to produce effects in the ontogeny of specific neurotransmitters as revealed by previous studies using different doses and analyses of Δ^9-THC concentrations in the plasma of cannabinoid-treated mothers and in the body of their pups (see Fernández-Ruiz et al., 1992; Bonnin et al., 1995, for more details). Control rats received vehicle (sesame oil) alone. The treatment was maintained daily until Day 24 after birth when the pups were weaned and separated by gender. Afterwards, they were left to mature for at least seven additional weeks. After a total period of at least 10 weeks after birth, animals of the four experimental groups (oil- or Δ^9-THC-exposed males or females) were divided in three different series: (i) one-third was sacrificed immediately before being used in operant morphine self-administration paradigms; (ii) a second third was subjected to analysis of operant morphine self-administration and sacrificed immediately after the last testing day; and (iii) the last third was subjected to analysis of operant morphine self-administration, but, after the last testing day, the animals were changed to saline to extinguish the morphine response and, 15 days later, they were sacrificed. In the three cases, after sacrifice, the brains were removed and rapidly frozen by immersion in a bath of methyl–butane cooled with dry ice. In an additional experiment, and using separate groups of animals, oil- and Δ^9-THC-exposed males and females were examined by operant food-reinforced behavior.

2.2. Operant food and morphine self-administration paradigms

2.2.1. Surgery

Subjects were surgically prepared with an intravenous catheter placed in the jugular vein. Polyvinylchloride tubing

(0.064 id) was implanted in the right jugular vein approximately at the level of the atrium under ketamine and diazepam anesthesia. The catheter was passed subcutaneously and exited in the midscapular region. The catheter then passed through a spring tether system (Alice King Chatham, Hawthorne, CA) that was mounted to the skull of the rat with dental cement. All subjects were housed individually following surgery and given at least 7 days to recover.

2.2.2. Apparatus

Twelve operant chambers (Coulbourn Instruments, Allentown, PA) were used for operant food-reinforced and morphine-self-administration studies. Two levers, designed to register a response when 3.0 g of force was applied, were placed 14 cm apart on the front wall of the chamber. A microliter injection pump (Harvard 22) was used to deliver intravenous saline or drug injections to the rat in the self-administration studies. Food and drug delivery, operant data acquisition, and storage were accomplished on IBM computers (MED Associates, St. Albans, VT).

2.2.3. Acquisition of morphine self-administration behavior under PR schedule of reinforcement

Δ^9-THC-exposed and control groups of both sexes were left during 15 days to self-administer morphine under a PR schedule of reinforcement described previously (Ambrosio et al., 1999). Under this schedule, the number of lever presses required to obtain a reinforcer is increased for successive reinforcers, following the series: 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, . . ., 901, . . ., etc., until the animal fails to meet the demands of the schedule. This is called the "breaking point." When the subject completed the successive demands of the PR schedule on the lever on the left-hand side of the chamber, a stimulus light turned on over the lever that signaled the drug delivery and resulted in administration of a dose of 1 mg/kg morphine sulfate. Lever presses on the lever on the right-hand side of the chamber were recorded, but they did not have programmed behavioral consequences. The drug was delivered in a 90- to 115-μl injection volume (dependent upon the weight of the subject) during a 15-s interval. A 30-s time out period in which responses had no scheduled consequence followed each drug delivery. Subjects were given access to the drug 12 h/day during the dark period of the light/dark cycle. Food and water were available throughout the entire experiment via a water bottle and food tray. The 12-h light/dark cycle was preserved via illumination of the chamber with a white house light.

2.2.4. Operant food-reinforced behavior

Before training, the body weight of animals of the four experimental groups was reduced until 90–95% of original body weight. This body weight reduction was held constant throughout the entire experiment. Initially, animals were habituated to the chamber components with an autoshaping program that randomly delivered a food pellet at an average of every 60 s during 30 min for 3 days. After this autoshaping period, acquisition of food-reinforced behavior under a fixed ratio 1 (FR1) schedule of reinforcement was studied during 6 days in 30-min sessions. A single lever press on the left-hand lever resulted in delivery of a food pellet (45-mg Noyes pellets, Middlesex, UK) and turned on a stimulus light over the lever that signaled pellet delivery. Lever presses on the right-hand lever had no programmed consequences. Requirements were subsequently raised to FR3, FR5, and FR10 in 60-min sessions. A programmed 30-s time out period in which responses had no programmed consequences followed each food pellet delivery. After the FR10 schedule was achieved, the same PR schedule of morphine self-administration was studied during 20 days in 3-h sessions. In each session, the PR program was shut off if the subject did not gain a food pellet during the last 30 min.

2.3. DA and DOPAC determinations

Brains from the four experimental groups in the three different series described above were used for manually obtaining coronal slices (around 500 μm thick) at two different levels containing the ventral tegmental area and the nucleus accumbens, according to Palkovits and Brownstein (1988). Subsequently, both structures were dissected and homogenized in 20–40 vol of cold 150 mM potassium phosphate buffer, pH 6.8. An aliquot of each homogenate was used for the analysis of protein concentration (Lowry et al., 1951), whereas the remaining homogenates were diluted (one-half) in ice-cooled 0.4 N perchloric acid containing 0.4 mM sodium disulfite and 0.90 mM EDTA. Dihydroxybenzylamine was added as an internal standard. The diluted homogenates were then centrifuged and the supernatants injected into the HPLC system to determine the contents of DA and DOPAC, according to our previously published method (González et al., 1999). Briefly, the HPLC system consisted of a Spectra-Physics 8810 isocratic pump. The column was a RP-18 (Spherisorb ODS-2; 125 × 4.6 mm, 5 μm particle size; Tecknokroma, Barcelona, Spain). The mobile phase consisted of 100 mM citric acid, 100 mM sodium acetate, 1.2 mM heptane sulfonate, 1 mM EDTA, and 7% methanol (pH 3.9), and the flow rate was 0.8 ml/min. The effluent was monitored with a coulochemical detector (Coulochem II, ESA) using a procedure of oxidation/reduction (conditioning cell: +360 mV; analytical cell 1: +50 mV; analytical cell 2: −340 mV). The signal was recorded from the analytical cell 2, with a sensitivity of 50 nA (10 pg/sample), on a Spectra-Physics 4290 integrator and the results were given as area under the peaks. Values were expressed as nanograms per milligram of protein.

2.4. Statistics

Total reinforcers per session (equal to ordinal breaking points) and average of total morphine intake were dependent

variables in the morphine-reinforced behavior. For the food-reinforced study, total pellet number per session was the dependent variable. A multivariate repeated measures analysis of variance (ANOVA) was used in all cases. Following a significant F value, Student–Newman–Keuls post hoc analysis was performed for assessing specific group comparisons. Calculations were performed using version 10.0 of the SPSS statistical package.

3. Results

3.1. Morphine self-administration behavior

The analysis of morphine self-administration behavior (under a PR schedule of reinforcement) in Δ^9-THC- or oil-exposed adult animals revealed that the most significant effect was on gender [$F(1,35) = 10.72$, $P < .05$] and it was independent of the perinatal treatment with Δ^9-THC [Δ^9-THC alone: $F(1,35) = 0.346$, ns; two-way interaction: $F(1,35) = 0.897$, ns]. Thus, female offspring showed a higher response rate than male offspring across the acquisition period and this occurred equally for the animals born from Δ^9-THC-treated mothers than for those born from vehicle-treated ones (Figs. 1 and 2). Breaking point numbers

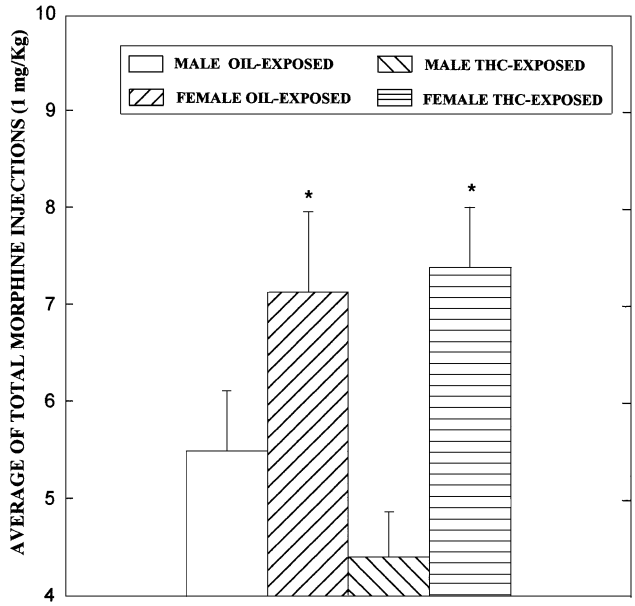

Fig. 2. Average of total morphine injections in adult males and females that had been perinatally exposed to Δ^9-THC or vehicle (details in the text). Values are expressed as means ± S.E.M. of at least six animals per group. Statistical differences were assessed by two-way (Gender × Δ^9-THC Treatment) ANOVA followed by the Student–Newman–Keuls test (* $P < .05$ versus males).

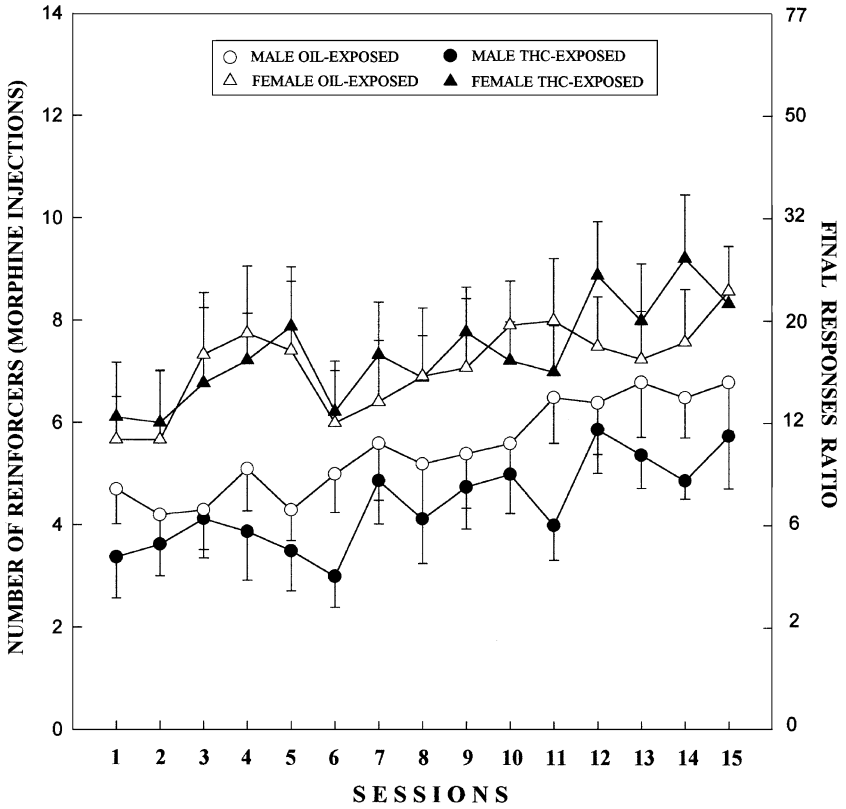

Fig. 1. Pattern of morphine self-administration in adult males and females that had been perinatally exposed to Δ^9-THC or vehicle (details in the text). Values are expressed as means ± S.E.M. of at least six animals per group and day. Statistical differences were assessed by multivariate (Gender × Δ^9-THC Treatment × Acquisition Day) ANOVA followed by the Student–Newman–Keuls test.

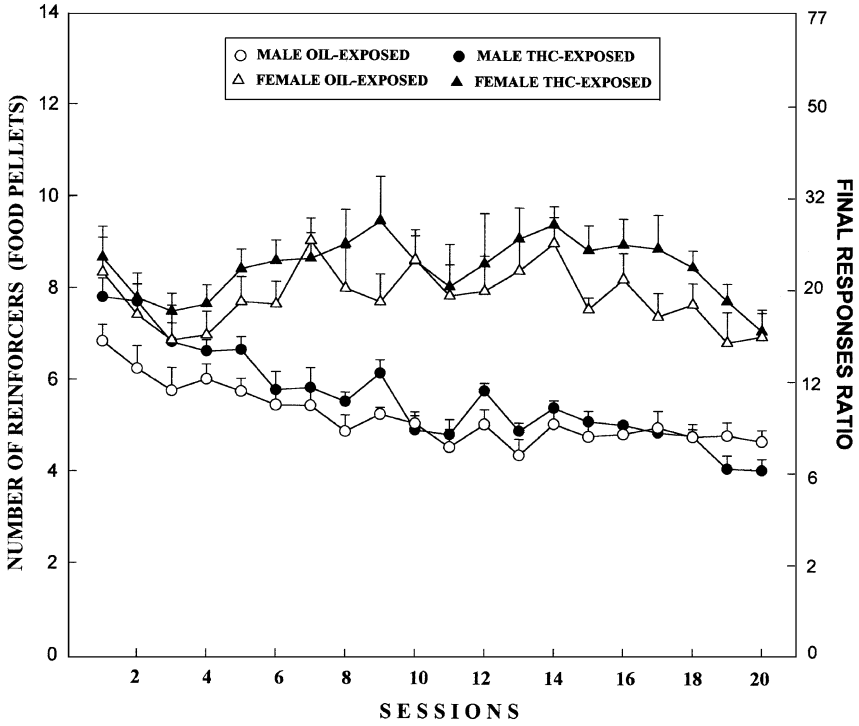

Fig. 3. Pattern of food-reinforced behavior in adult males and females that had been perinatally exposed to Δ^9-THC or vehicle (details in the text). Values are expressed as pellet number earned per 100 g of body weight and correspond to means ± S.E.M. of at least six animals per group and session. Statistical differences were assessed by multivariate (Gender × Δ^9-THC Treatment × Session) ANOVA followed by the Student–Newman–Keuls test.

were also significantly higher in female than male offspring of both Δ^9-THC- and vehicle-treated mothers (Fig. 1).

3.2. Food-reinforced behavior

Similarly to morphine self-administration behavior, there were significant differences between genders [$F(1,20) = 72.32$, $P < .0005$] for food-reinforced behavior, but they were not affected by the perinatal Δ^9-THC treatment [Δ^9-THC alone: $F(1,20) = 2.78$, ns; two-way interaction: $F(1,20) = 0.14$, ns] (see Fig. 3 for details). Breaking point numbers for food reward in the adult females were significantly higher than in adult males, for both Δ^9-THC- and vehicle-treated rats across all the session, except second and third sessions.

3.3. Neurochemical determinations

The analysis of DOPAC/DA ratio, a frequently used index for the activity of dopaminergic neurons (for review, see Moore et al., 1987), revealed that there is an effect of the perinatal Δ^9-THC treatment [nucleus accumbens: $F(1,24) = 3.85$, $P < .05$; ventral tegmental area: $F(1,20) = 5.91$, $P < .05$], but interacting with gender [two-way interaction: nucleus accumbens: $F(1,24) = 3.89$, $P < .05$; ventral tegmental area: $F(1,20) = 4.42$, $P < .05$]. However, this only oc-

Table 1
DOPAC/DA ratio in the ventral tegmental area and the nucleus accumbens of male and female adult rats that had been perinatally exposed to Δ^9-THC or vehicle from the fifth day of gestation up to weaning on Day 22 after birth (details in the text)

Brain region	Testing day	Females		Males	
		+ Oil	+ Δ^9-THC	+ Oil	+ Δ^9-THC
Nucleus accumbens	premorphine	0.36 ± 0.03	0.26 ± 0.03 *	0.26 ± 0.03	0.26 ± 0.05
	postmorphine	0.23 ± 0.03	0.22 ± 0.02	0.25 ± 0.03	0.22 ± 0.04
	extinction	0.18 ± 0.01	0.16 ± 0.02	0.19 ± 0.02	0.22 ± 0.03
Ventral tegmental area	premorphine	0.29 ± 0.03	0.22 ± 0.01 #	0.30 ± 0.02	0.35 ± 0.04
	postmorphine	0.25 ± 0.03	0.27 ± 0.02	0.27 ± 0.01	0.29 ± 0.05
	extinction	0.26 ± 0.03	0.22 ± 0.06	0.31 ± 0.06	0.39 ± 0.06

Values are means ± S.E.M. of five to six determinations per group. Data were assessed by two-way ANOVA (Gender × Perinatal Treatment) followed by the Student–Newman–Keuls test.

* $P < .05$ versus the corresponding oil-exposed group.

\# $P < .05$ versus the other three groups.

curred in the first series of animals tested, i.e., before being subjected to morphine self-administration. The post hoc analysis revealed that DOPAC/DA ratio in the nucleus accumbens of Δ^9-THC-exposed females was lower than that for oil-exposed females and similar to oil- and Δ^9-THC-exposed males (Table 1). A similar reduction occurred in the ventral tegmental area of Δ^9-THC-exposed females compared to oil-exposed females and also compared to oil- and Δ^9-THC-exposed males (Table 1). All these changes, however, disappeared after 15 days of daily sessions of morphine self-administration and they did not reappear again after 15 additional days of extinction of this response. However, a tendency of DOPAC/DA ratio to decrease could be, in general, appreciated in the nucleus accumbens of the four groups after the period of morphine self-administration and, in particular after the extinction of the response (see Table 1). This is concordant with the reduction in basal mesolimbic dopaminergic activity found in morphine-dependent rats (Spanagel et al., 1994). No changes were seen in basal ganglia structures (data not shown).

4. Discussion

Overall, these results suggest that the perinatal Δ^9-THC exposure does not affect the reinforcing efficacy of morphine and food in a PR schedule. This contrasts with our previous finding using an FR1 schedule (Vela et al., 1998), in which the perinatal exposure to Δ^9-THC at the same dose used here increased morphine self-administration behavior in females but not in males. As mentioned in the Introduction, differences in the response requirements of both reinforcement schedules might explain these apparently conflicting observations. Thus, an FR schedule reveals if a drug is reinforcing or not, but the reinforcing efficacy of this drug needs to be tested in a PR schedule (for review, see Arnold and Roberts, 1997). So, with our previous results (Vela et al., 1998) and the present ones and always considering the limitations of using a single dose of Δ^9-THC in both studies, we might conclude that morphine is particularly preferred by adult females that had been perinatally exposed to Δ^9-THC (Vela et al., 1998), but this vulnerability to morphine disappeared if we forced the animals to work harder to get the opiate.

Another observation from the present study is that gender may be a more important factor than the perinatal Δ^9-THC exposure, in terms of influencing the reinforcing properties of morphine, at least under the conditions of the present experiment. The same result was obtained here for a natural reinforcer such as food, and similar findings have been reported in studies examining other drugs of abuse, where a marked sexual dimorphism was always evident (for a recent review, see Campbell and Carroll, 2000). For instance, females that were exposed to cocaine during perinatal life responded more intensely than males to this drug or to other psychostimulants in adulthood (Dow-Edwards, 1989; Hughes et al., 1991; Lin and Kellogg, 1996). The molecular basis underlying this sexual dimorphism has not been elucidated yet, but some hypotheses deserve to be mentioned. For instance, it has been shown that estrogens modify μ-opioid receptor binding in various brain regions of ovariectomized rats (Piva et al., 1995) and that the density of this receptor fluctuates during the ovarian cycle (Martini et al., 1989), so potential interactions between sex steroid hormones and the endogenous opioid system might account for gender-dependent differences in morphine self-administration behavior. Female rats usually exhibit a higher spontaneous locomotor activity compared to male rats (Hyde and Jerussi, 1983), and this might also account for the sex differences in the reinforcing efficacy of morphine. In support of this, we have shown, using a similar PR schedule of morphine self-administration, that Lewis rats, which have a higher spontaneous locomotor activity than Fischer 344 rats, reached higher daily average breaking points and final response ratios (Martín et al., 1999). A last explanation could be based on the existence of gender-dependent differences in the basal activity and/or sensitivity of mesolimbic dopaminergic neurons, which are relevant components of the reward circuitry (Spanagel and Weiss, 1999). In this study, we have measured DOPAC/DA ratio, which has been largely used as an index of neuronal activity (Moore et al., 1987), and we observed that this parameter was higher in the nucleus accumbens (where the terminals of mesolimbic dopaminergic neurons are clustered) of females than males. Therefore, it would be tempting to assume that this higher mesolimbic dopaminergic activity might enable females to respond more intensely to morphine or food as compared to males because of the key role played by this dopaminergic pathway in reinforcement processes (Spanagel and Weiss, 1999).

Interestingly, DOPAC/DA ratio was reduced in the nucleus accumbens and the ventral tegmental area of Δ^9-THC-exposed female rats, which is concordant with previous observations obtained with animals exposed to hashish extracts (Navarro et al., 1996). However, this had no influence in the degree of vulnerability to morphine (and also in the rewarding properties of food). Possibly, this might mean that changes in DOPAC/DA are not the only variable to predict a change in morphine self-administration. It could be argued that the changes in dopaminergic activity might affect the reinforcer efficacy of morphine in an FR schedule of self-administration, as we reported previously (Vela et al., 1998), but this would have no influence in a PR schedule, where the animal has to work harder to get the morphine. In other words, the changes in dopaminergic activity might account for the acute reinforcing effect of morphine, but other elements of the reward system, mainly opioidergic neurons, might be crucial for maintenance of the morphine self-administration, particularly in regard to the constraints of a PR schedule (for a recent review, see Koob, 2000).

It is also noteworthy to mention that these changes in DOPAC/DA ratio appeared prior to morphine self-adminis-

tration but disappeared after 15 days of chronic access to the opioid and they did not appear again despite the extinction of this behavior. In general, a tendency of the DOPAC/DA ratio to decrease was observed during the phases of morphine self-administration behavior and extinction of the response, which is in concordance with results published by other authors for morphine dependence and withdrawal (Spanagel et al., 1994; Tokuyama et al., 2000). This tendency was seen in the nucleus accumbens, but not in the ventral tegmental area, and was relatively similar for the four groups tested here.

In summary, our data using a PR schedule for operant food- and morphine-reinforced behavior are indicative that females respond more intensely than males. Our data also indicate that, when rats are forced to work harder for the reward, the exposure to Δ^9-THC is not a factor influencing the response to both reinforcers. The neurochemical analysis revealed that the activity of limbic dopaminergic neurons, prior to morphine self-administration, was higher in females than males, and that the perinatal Δ^9-THC treatment reduced the activity of these neurons only in females, although this had no influence on morphine vulnerability in these animals.

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