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Perinatal exposure to Δ^9-tetrahydrocannabinol increases presynaptic dopamine D_2 receptor sensitivity: a behavioral study in rats

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Abstract

The endogenous cannabinoid system is a relevant modulator of dopaminergic synapses in dorsal striatum. Perinatal exposure to cannabinoid receptor agonists has been described to affect the development of dopaminergic circuits in rat brain. The epigenetic alterations described affected both dopamine neurons and dopamine receptor-expressing neurons. The present work has been designed to explore the effects of maternal exposure to orally delivered Δ^9-tetrahydrocannabinol, (Δ^9-THC 0.1, 0.5, 2 mg/kg) on the behavioural responses to the dopamine receptor agonists apomorphine (0.1 mg/kg) and quinpirole (0.5 mg/kg), at doses that target presynaptic dopamine D2 receptors. Maternal exposure to Δ^9-THC affected both the developmental pattern of motor behaviours, and the behavioural responses to acute injections of apomorphine and quinpirole, tested in an open field. The effects were sex dimorphic, being more intense in male animals. Perinatal exposure to Δ^9-THC resulted in enhanced presynaptic dopamine D2 receptor mediated responses such as immobility and inhibition of locomotion. Additionally, postsynaptic dopamine D2 receptor agonist-induced stereotypes were reduced in the group exposed to the highest dose of Δ^9-THC (2 mg/kg). However, the late-onset pattern of behavioural activation observed after acute quinpirole exposure was equal in vehicle- and cannabinoid-treated animals. These effects suggest that perinatal exposure to Δ^9-THC affects the functionality of dopaminergic autoreceptors, inducing a greater sensitivity to the presynaptic actions of dopamine D_2 receptor agonists.

Keywords: Cannabinoid; Perinatal exposure; Behaviour; Dopamine receptors; Apomorphine; Quinpirole

1. Introduction

Maternal drug abuse has been found to induce long-term dysfunctions in neural development in both humans (Day et al., 1994; Levitt, 1998; Robins and Mills, 1993; Zuckerman et al., 1989) and animal models (Navarro et al., 1994b, 1995; Navarro and Rodriguez de Fonseca, 1998; Rodriguez de Fonseca et al., 1991; Rubio et al., 1998). Among drugs used in pregnancy *Cannabis sativa* preparations remain as the most widely used illicit drugs (Gardner and Lowinson, 1991; Gurnack and Paul, 1997). The consumption of marihuana by women during the perinatal period affects neurobehavioural development of their children because the psychoactive principles of *C. sativa* cross the placental barrier during the gestation (Hutchings et al., 1989) and are transferred to the maternal milk along the lactation (Jakubovic et al., 1997). Data from a longitudinal study in children born from marihuana smokers support this hypothesis (Fried and Smith, 2001). The target of psychoactive cannabinoids is the endogenous brain cannabinoid system (ECS). It includes the cannabinoid receptor CB_1 (Devane et al., 1988, Matsuda et al., 1990; Howlet, 1995) and the endogenous ligands anandamide and 2-arachydonyl glycerol (Devane et al., 1992; Mechoulam et al., 1995; Sugiura et al., 1995). The endogenous cannabinoid system seems to be present since early stages of life, playing a relevant role in brain organization (Fernandez-Ruiz et al., 2000). Thus, several studies have described the presence of the endogenous ligands anandamide and 2-arachydonyl glycerol

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(Berrendero et al., 1999) and the cannabinoid CB_1 receptors in the developing brain (Rodriguez de Fonseca et al., 1993).

Previous studies have shown in rodents that perinatal exposure to the main psychoactive constituent of *C. sativa*, Δ^9-tetrahydrocannabinol (Δ^9-THC), might interfere, acting as an epigenetic factor, with the rigidly ordered temporal sequences of events that occur during the ontogeny of the central nervous system (Navarro et al., 1994b, 1995; Navarro and Rodriguez de Fonseca, 1998; Walters and Carr, 1986, 1988). Therefore, cannabinoid exposure during development produces long term effects modifying the functional regulation of motor behaviours, cognition, learning, and memory process, as well as nociception (Fride and Mechoulam, 1996; Fried and Smith, 2001; Navarro et al., 1996).

A specific target of neurodevelopmental alterations induced by cannabinoids are dopaminergic neurons (Rodriguez de Fonseca et al., 1992a). Experimental approaches to cannabinoid dopamine interactions have provided evidence for a functional and anatomical link between these two systems that might be relevant for the understanding of dopamine-related disorders such as stress, addiction and psychosis (Navarro et al., 1993b; Rodriguez de Fonseca et al., 1992a,b, 1994). Moreover, Cannabis consumption has been related with mental disorders such as schizophrenia (Andreasson et al., 1987; Gorriti et al., 1999). It is noteworthy that cannabinoid exposure is associated with both dysfunctions of motor behaviours and alterations in the activity of nigrostriatal and mesolimbic dopaminergic neurons (Navarro et al., 1993a, 1994a). The described alterations of developing monoaminergic system include a reduction of the expression and activity of tyrosine hydroxylase and changes in the ontogenic profile of dopamine D_1 and D_2 receptors (Bonnin et al., 1995; Fernandez-Ruiz et al., 1994; Rodriguez de Fonseca et al., 1992b; Suarez et al., 1999).

In an attempt to further understand the nature of Δ^9-THC-induced changes in dopamine-regulated behaviours, we have studied the behavioural responses derived from dopamine D_2 receptor stimulation in animals perinatally exposed to low doses of the cannabinoid, relevant for human consumption. This study is relevant since cannabinoid CB_1 receptors and endocannabinoids are considered as key elements in synaptic transmission, both as regulators of presynaptic release of transmitters and postsynaptic modulators of input signalling (Di Marzo et al., 1998; Schlicker and Kathmann, 2001). Hypothetically, the stimulation of cannabinoid CB_1 receptors in developing synapses might permanently modify this role of "synaptic modulation" and ultimately might lead to disturbances in motor behaviours. To achieve this goal, we have developed a study to explore the effects of maternal exposure to low doses of Δ^9-THC (0.1, 0.5, 2 mg/kg orally from the 5th day of gestation to the 24th of lactation), on the behavioural responses to the dopamine receptor agonists apomorphine (0.1 mg/kg sc) and quinpirole (0.5 mg/kg sc).

2. Materials and methods

2.1. Subjects

Female virgin rats of the Wistar strain (>8 weeks old; 200–250 g) were housed in a room with controlled photoperiod (lights on from 8:00 a.m. to 8:00 p.m.) and temperature (23 ± 1 °C). They were habituated to handling for 1 week before starting the experiments, with free access to standard food (Panlab, Barcelona) and water. Daily vaginal smears were taken and only those animals exhibiting three o 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 new vaginal smear was taken in the next day. Those animals showing the presence of sperm cells were accepted as probably pregnant and used for the Δ^9-THC exposure studies. The day on which sperm plugs were found was designated as the first day of gestation. From that day animal weight, water and food intake were measured daily up to the day of weaning. Pregnant females received a daily oral dose of Δ^9-THC (0.1, 0.5 or 2 mg/kg) or vehicle (sesame oil) from the 5th day of gestation until 24th day of lactation. After weaning, the animals were separated and housed, 4–5 animals of the same sex and treatment per cage. In short, 192 animals were used for behavioural studies, distributed as follows: vehicle–sesame oil (59), 0.1 mg/kg Δ^9-THC (49), 0.5 mg/kg Δ^9-THC (45), 2 mg/kg Δ^9-THC (39). They were studied at adult age (>70 days). Studies in female animals were performed after ovariectomy to avoid the effects of the oestrous cycle. All procedures were carried out according to European Communities directive 86/609/EEC regulating animal research.

2.2. Drugs

Δ^9-THC of greater than 95% purity was provided in an ethanol solution by the National Institute on Drug Abuse (NIDA), USA. Immediately before use, the ethanol was evaporated under a nitrogen flow and the residue was emulsified in sesame oil as vehicle. Pregnant females received a daily single oral dose of Δ^9-THC (0.1, 0.5, or 2 mg/kg bodyweight) or vehicle (sesame oil) in a volume of 0.1 ml. The doses of Δ^9-THC chosen were an extrapolation from currents estimates of low to moderate exposure of this compound in humans, and were corrected, considering the differences in route of administration and body surface area (Rosenkrantz et al., 1975).

Apomorphine (0.1 mg/kg) and quinpirole (0.5 mg/kg) were obtained through TOCRIS, Cookson, UK. Both drugs were prepared in sterile 0.9% saline, and were administered subcutaneously in a volume of 1 ml/kg.

Table 1
Effects of perinatal Δ^9-THC exposure on several gestational and lactational parameters

Parameters	Mother treatment Δ^9-THC			
	+Oil	+0.1 mg/kg	+0.5 mg/kg	+2 mg/kg
Mother food intake (g)				
Gestation	20.60 ± 0.46	20.95 ± 0.34	21.40 ± 0.74	22.10 ± 0.62
Lactation	53.25 ± 4.56	54.33 ± 3.89	57.83 ± 4.50	57.66 ± 4.30
Mother water intake (ml)[a]				
Gestation	37.50 ± 1.26	34.70 ± 1.08	39.36 ± 1.05	41.36 ± 1.50
Lactation	72.91 ± 5.75	70.20 ± 5.61	79.16 ± 6.50	82.16 ± 6.60
Mother weight gain (g)[b]	115.20 ± 3.03	110.00 ± 5.60	115.75 ± 6.28	122.33 ± 18.61
Gestational length (days)	22.60 ± 0.24	22.00 ± 0.00	22.75 ± 0.29	22.33 ± 0.41
Litter size	11.80 ± 0.89	12.25 ± 0.73	11.25 ± 0.99	13.00 ± 0.71
Litter weight (g)	79.50 ± 4.10	75.90 ± 2.80	79.10 ± 5.60	87.40 ± 8.03
No. males	6.00 ± 1.32	6.50 ± 1.11	5.50 ± 0.33	7.33 ± 1.08
No. females	5.80 ± 0.89	5.75 ± 1.19	5.75 ± 1.09	5.67 ± 1.47
Postnatal Mortality	0.60 ± 0.67	0.00 ± 0.29	0.00 ± 0.00	0.67 ± 0.82

Values are means ± S.E.M. of mothers and litter per treatment. Statistics were assessed by analysis of variance.
[a] Average value per day.
[b] Difference between the weight in the day before delivery and the weight before mating.

2.3. Behavioural studies

The study was performed with an open-field apparatus. It consisted in a square of 40 × 35 × 35 cm, equipped with photocells beams spaced 10 cm from one another in their walls. The test started placing the rat in an open field and was observed during three intervals of 5 min each; immediately after the injection, 60 min after the injection and 120 min after the injection. Trained observers, blind to the experimental conditions, assessed the behavioural act of *Immobility*, defined as the time spent by the animals in absolute quietness (measured in seconds) and *Stereotypes*, counted as repetitive behavioural acts (without considering the initial one). *Locomotion* was automatically registered by means of a photocells system in the cage (measured as number of crossings).

2.4. Data analysis

Data recorded (from each 5-min interval) were evaluated by multifactorial analysis of variance (Pretreatment × Treat-Treatment interaction effect). When a significant F value was found, post hoc analysis (Tukey) was performed for assessing specific group comparisons. Calculations were performed using the SPSS statistical package.

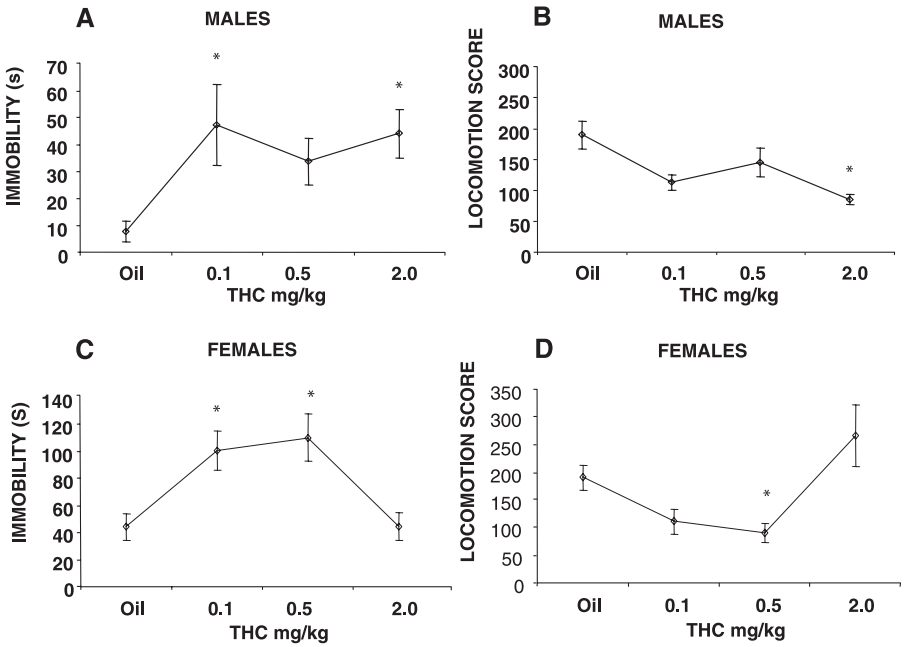

Fig. 1. Perinatal exposure to Δ^9-THC modifies the behavioural responses to an open field in the adulthood. A and C: immobility measured in seconds in males and females after the injection of vehicle (saline, subcutaneous). B and C: locomotion measured in crossings after the injection of vehicle (saline, subcutaneous). Data are means ± S.E.M. of accumulated values measured in 12 animals per group. * $P < .05$, vs. vehicle–oil-pretreated animals.

2.5. Experimental designs

2.5.1. Experiment 1: perinatal Δ^9-THC exposure in adult rats

The first experiment studied the spontaneous behavioural performance in the open field in animals perinatally treated with Δ^9-THC. Rats perinatally pretreated with 0.1, 0.5 or 2 mg/kg of Δ^9-THC were studied in the adult period after the acute subcutaneous administration of saline. The animals were placed in the apparatus and were observed for 5 min immediately, 60 min and 120 min after the injection.

2.5.2. Experiment 2: perinatal Δ^9-THC exposure and behavioural responses to apomorphine in adult rats

The acute behavioural actions of the administration of the dopamine receptor agonist apomorphine were studied in the open field in animals perinatally pretreated with Δ^9-THC. Each experimental group (sesame oil, 0.1, 0.5, 2 mg/kg) was divided in two. The first group ($n = 12$) received a subcutaneous injection of vehicle (saline) and the second group ($n = 12$) received a subcutaneous injection of apomorphine (0.1 mg/kg). After the injection, each animal was placed in the open field and was observed for 5 min immediately, 60 min and 120 min after the injection.

2.5.3. Experiment 3: perinatal Δ^9-THC exposure and behavioural responses to quinpirole in adult rats

Behavioural responses derived of acute administration of the dopamine D_2 receptor agonist quinpirole were evaluated as described for apomorphine. Each perinatal Δ^9-THC-exposed group (sesame oil, 0.1, 0.5, 2 mg/kg) was divided in two. The first group ($n = 12$) received a subcuta-

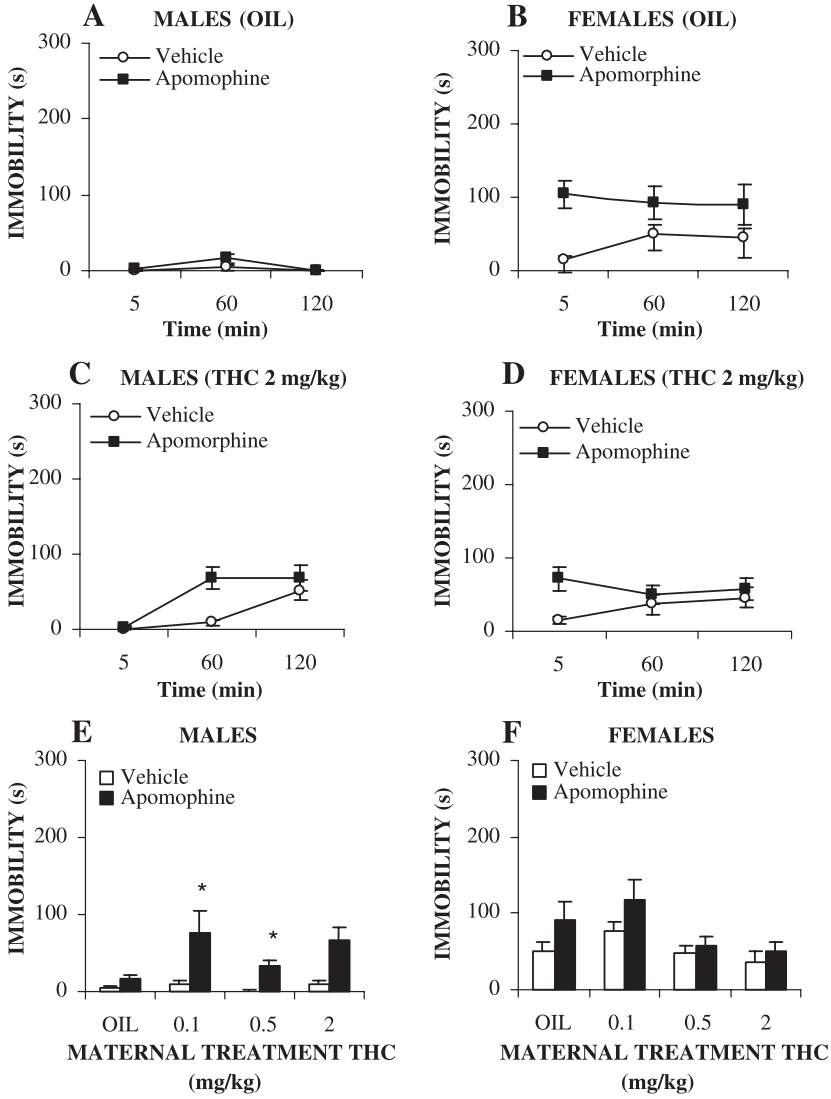

Fig. 2. Perinatal exposure to Δ^9-THC modifies the behavioural responses to a to a low dose of apomorphine (0.1 mg/kg sc), measured in the open field. A and B: time-course of the immobility (in seconds) in oil-exposed males and females. C and D: time-course of the immobility (in seconds) in Δ^9-THC (THC, 2 mg/kg)-exposed males and females. E and F: immobility (in seconds) in all experimental groups, measured 60 min after apomorphine or vehicle injection. Data are means ± S.E.M. of values measured in 12 animals per group. $^*P < .05$, vs. vehicle–saline-treated animals.

neous injection of vehicle (saline) and the second group ($n = 12$) received a subcutaneous injection of quinpirole (0.5 mg/kg). After the injection, each animal was placed on the open-field and was observed for 5 min immediately, 60 min and 120 min after the injection.

3. Results

3.1. Effects of perinatal exposure to Δ⁹-THC on several gestational and lactational parameters

To assess the possible toxic effects of Δ^9-THC perinatal administration, we recorded several parameters throughout the gestation and the lactation (Table 1). Our results did not indicate major changes derived of cannabinoid exposure. During the gestation and lactation period, the average of food intake (per day) and the average of water intake (per day) were higher in rats treated with Δ^9-THC. Moreover, the average of mother weight gain (the difference between the weight in the day before delivery and the weight before mating) was highest in mothers exposed to the highest dose of Δ^9-THC (2 mg/kg). Both these effects may derive from the hyperphagic effects of cannabinoids (Williams and Kirkham, 1999).

3.2. Effects of perinatal exposure to Δ⁹-THC on the adult period (Fig. 1)

Animals perinatally exposed to Δ^9-THC exhibited a differential pattern of behaviour in the open field when compared to oil-exposed ones. Although along the first 5-min interval of study all animals exhibited similar patterns of locomotion and immobility (data not shown), THC-

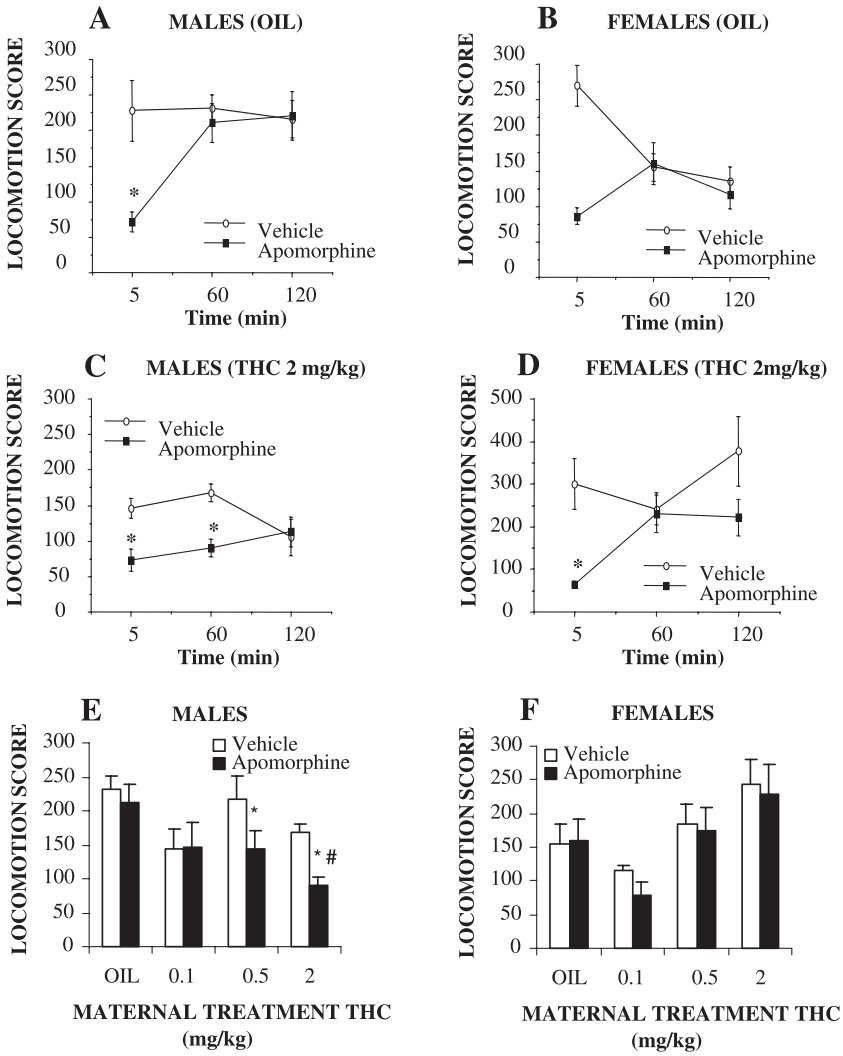

Fig. 3. Perinatal exposure to Δ^9-THC modifies the behavioural responses to a low dose of apomorphine (0.1 mg/kg sc), measured in the open field. A and B: time-course of the locomotion (crossings) in oil-exposed males and females. C and D: time-course of the locomotion (crossings) in Δ^9-THC (THC, 2 mg/kg)-exposed males and females. E and F: locomotion (crossings) in all experimental groups, measured 60 min after apomorphine or vehicle injection. Data are means ± S.E.M. of values measured in 12 animals per group. $^*P < .05$, vs. vehicle–saline treated animals; $^{\#}P < .05$, vs. oil–quinpirole-treated animals.

exposed animals were less active along the second (60 min) and third (120 min) interval of study. Illustrating this finding, Fig. 1 shows locomotor activity and immobility 120 min after saline injection. Male and female rats perinatally exposed to 0.1 and 2 mg/kg of Δ^9-THC exhibited an increase in the time spent in immobility (dose–effect, $F(3,76)=3.8$, $P<.012$ and $F(3,78)=7.16$, $P<.001$, respectively) compared to the vehicle–oil ones Fig. 1A and C. According to this result in immobility, we observed a similar decrease in locomotion in Δ^9-THC-exposed males and females, when compared to oil-exposed ones (dose–effect, $F(3,54)=4.1$, $P<.01$ and $F(3,78)=7.2$, $P<.001$, respectively). (Fig. 1B and D). The results in the highest dose of Δ^9-THC (2 mg/kg) were more accentuated in males but disappeared in females, a sex-dimorphic response already described after perinatal exposure to cannabinoids (Navarro et al., 1995).

3.3. Effects of acute apomorphine on Δ^9-THC perinatal exposure (Figs. 2 and 3)

Apomorphine induced a time-dependent alteration in motor activity in both males and females, characterized mainly by a short-lasting reduction in activity that normalizes within the first 60 min (Fig. 3A and B). The administration of apomorphine resulted in an increase of immobility in the Δ^9-THC pretreated males, but not females, (Pretreatment × Treatment interaction effect, $F(7,73)=3.4$, $P<.003$ and $F(7,76)=1.8$, $P<.08$, respectively) when compared to the vehicle–saline-treated groups (Fig. 2E and F). It also resulted in a potentiation of apomorphine-induced decrease in locomotion as showed in Fig. 3C and E, when compared to both vehicle–saline-treated groups (Pretreatment × Treat-Treatment interaction effect, $F(7,70)=3.1$, $P<.007$), and oil–apomorphine-treated groups (dose–effect, $F(3,35)=$

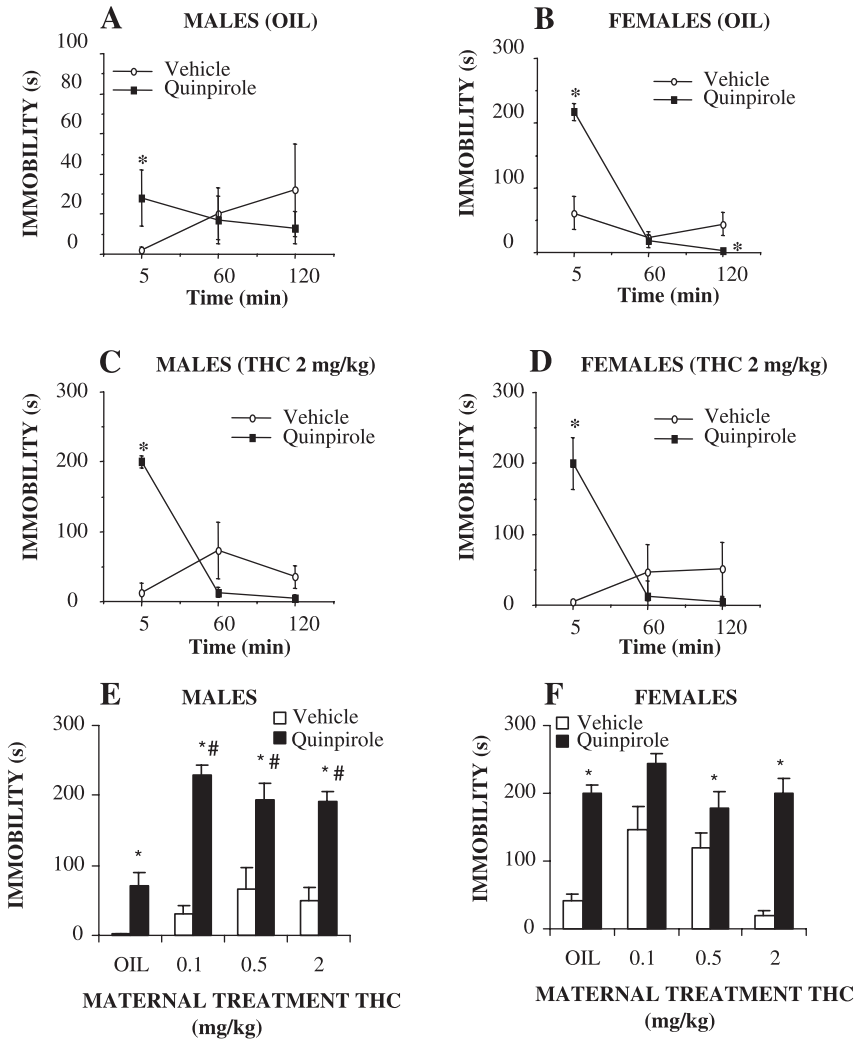

Fig. 4. Perinatal exposure to Δ^9-THC modifies the behavioural responses to a dose of the dopamine agonist quinpirole (0.5 mg/kg sc), measured in the open field. A and B: time-course of the immobility (in seconds) in oil-exposed males and females. C and D: time-course of the immobility (in seconds) in Δ^9-THC (THC, 2 mg/kg)-exposed males and females. E and F: immobility (in seconds) in all experimental groups, measured 5 min after quinpirole or vehicle injection. Data are means ± S.E.M. of values measured in 12 animals per group. * $P<.05$, vs. vehicle–saline treated animals; $^{\#}P<.05$, vs. oil–quinpirole treated animals.

2.7, $P < .058$).These effects appeared as a prolonged immobility and reduced locomotion observed in the second interval (60 min) when activity normalizes in apomorphine-treated controls. These effects were not observed in female rats (Fig. 3D and F).

3.4. Effects of acute quinpirole on Δ^9-THC perinatal exposure (Figs. 4–6)

Behavioural effects of the acute treatment with the dopamine D_2 receptor agonist quinpirole (0.5 mg/kg), measured at 5, 60 and 120 min after the injection, are depicted in Figs. 4–6. Perinatal exposure to Δ^9-THC resulted in an increased sensitivity effect to the immobility induced by quinpirole in the early phase of the study, 5 min after injection, when compared to the vehicle–saline-treated groups (Pretreatment × Treatment interaction effect, $F(7,73) = 16.8$, $P < .000$), and compared to the oil–quinpirole-treated group (dose–effect, $F(3,38) = 12.9$, $P < .000$) (Fig. 4A, C and D). This effect disappeared thereafter, and was present only in males. In females, quinpirole-induced immobility was equal in all experimental groups, with the exception of the lowest dose of maternal Δ^9-THC, (dose–effect, $F(3,36) = 2.58$, $P < .069$) (Fig. 4B, D and F). These effects were observed also on locomotion: The administration of quinpirole resulted in a reduction in locomotor activity during the first 5 min of

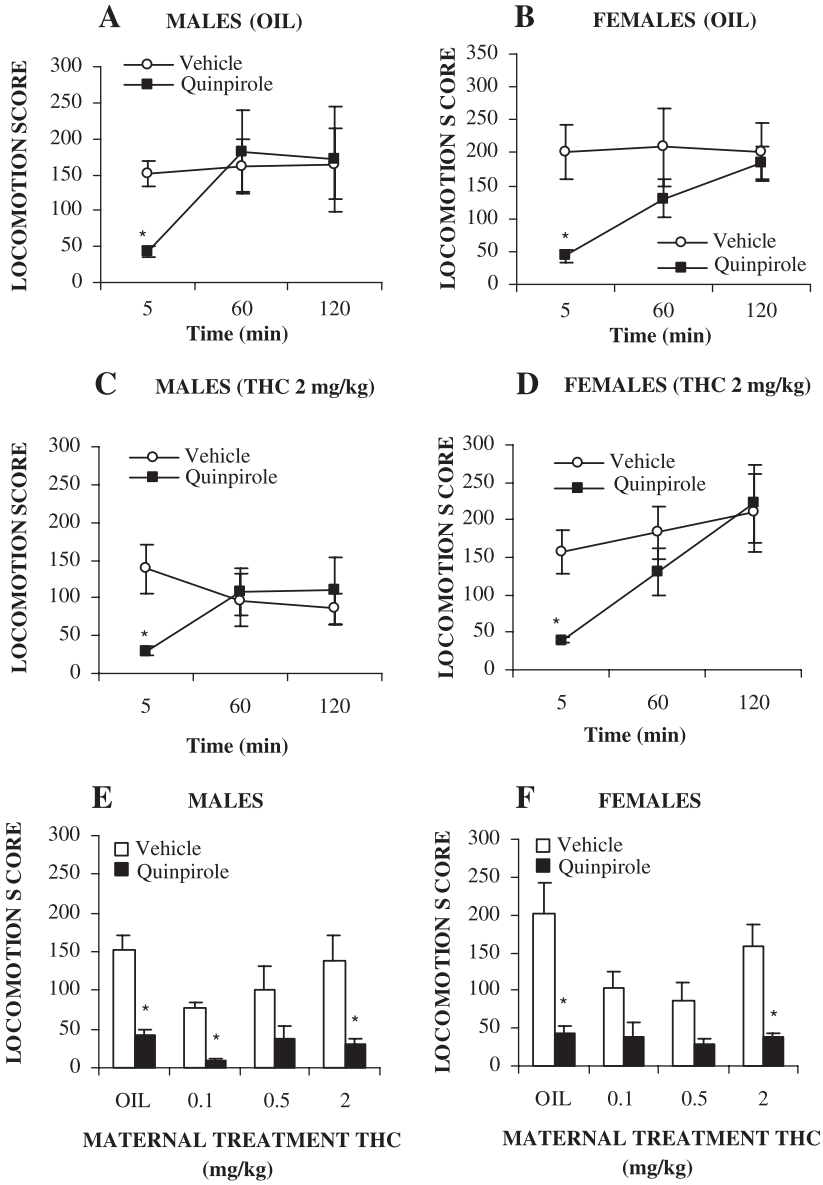

Fig. 5. Perinatal exposure to Δ^9-THC modifies the behavioural responses to a dose of the dopamine agonist quinpirole (0.5 mg/kg sc), measured in the open field. A and B: time-course of the locomotor activity (crossings) in oil-exposed males and females. C and D: time-course of the locomotor activity (crossings) in Δ^9-THC (THC, 2 mg/kg)-exposed males and females. E and F: locomotor activity (crossings) in all experimental groups, measured 5 min after quinpirole or vehicle injection. Data are means ± S.E.M. of values measured in 12 animals per group. * $P < .05$, vs. vehicle–saline treated animals.

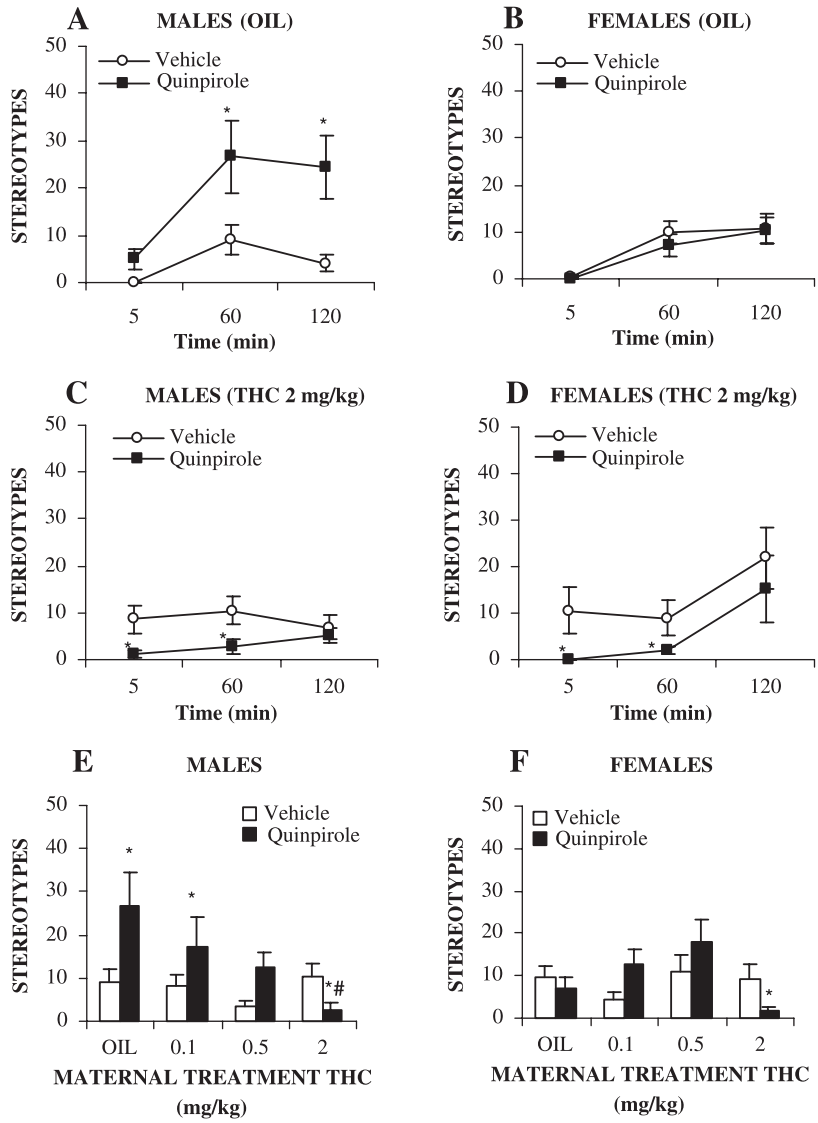

Fig. 6. Perinatal exposure to Δ^9-THC modifies the behavioural responses to a dose of the dopamine agonist quinpirole (0.5 mg/kg sc), measured in the open field. A and B: time-course of the stereotypes induced by quinpirole in oil-exposed males and females. C and D: time-course of the stereotypes induced by quinpirole in Δ^9-THC (THC, 2 mg/kg)-exposed males and females. E and F: stereotypes induced by quinpirole in all experimental groups, measured 60 min after quinpirole or vehicle injection. Data are means ± S.E.M. of values measured in 12 animals per group. $^*P<.05$, vs. vehicle–saline treated animals; $^{\#}P<.05$, vs. oil–quinpirole-treated animals.

study, when compared to the vehicle–saline-treated groups (Pretreatment × Treatment interaction effect, $F(7,70)=7.4$, $P<.000$), and a slight trend to a decrease compared to the quinpirole treated groups (dose–effect, $F(3,38)=2.5$, $P<.077$) (Fig. 5E). In females, locomotion score was reduced in all experimental groups 5 min after the administration of quinpirole when compared to vehicle–saline-treated groups (Pretreatment × Treatment interaction effect, $F(7,73)=7.81$, $P<.000$) (Fig. 5F).

However, postsynaptic effects of the D_2 receptor agonist were not enhanced in the animals exposed to Δ^9-THC: the stimulatory actions of quinpirole on locomotion observed from 60 and 120 min after the injection was normal in both oil- and cannabinoid-exposed rats (Fig. 5A–D). Moreover, quinpirole-induced stereotyped activity was decreased in a dose-dependent fashion in Δ^9-THC-exposed males, at 60 min (dose–effect, $F(3,38)=3.2$, $P<.032$) and at 120 min [dose–effect, $F(3,38)=2.9$, $P<.049$] postinjection (Fig. 6A, C and E). In female rats, only the group exposed to the highest dose of Δ^9-THC (2 mg/kg) showed a decrement in stereotypes after the administration of quinpirole, at 60 min [dose–effect, $F(3,36)=4.46$, $P<.009$], and there was no effect at time of 120 min [dose–effect, $F(3,36)=1.69$, $P<.186$] (Fig. 6B, D and F). These results indicate that perinatal exposure to Δ^9-THC results in either an increased sensitivity to the presynaptic inhibition driven by the stimulation of dopamine D_2 autoreceptors or a decreased postsynaptic sensitivity of dopamine D_2 receptors. The lack of consistent effects of quinpirole-induced hyperlocomotion further supports the first hypothesis.

4. Discussion

The results show that perinatal administration of Δ^9-THC lead to permanent alterations in behavioural patterns as revealed by the significant increase in spontaneous immobility and a decrement in locomotion in the open field (Fig. 1), as well as by the altered response to dopaminergic agonists of adult animals perinatally exposed to the cannabinoid (Figs. 2–6). The existence of psychomotor disturbances after perinatally cannabinoid exposure has been demonstrated previously by different authors, reporting a suppressant effect in motor behaviours in the adulthood in males and a psychomotor activation in females, when born from mothers exposed to relatively high doses of cannabinoids (Fernandez-Ruiz et al., 2000; Fride and Mechoulam, 1996; Navarro et al., 1994a,b, 1995). The novelty of the present studies is centred in the use of very low doses of Δ^9-THC, relevant for human consumption. Although as suggested in previous studies (Fernandez-Ruiz et al., 1994; Navarro et al., 1993a; Rodriguez de Fonseca et al., 1991), the alterations on the behavioral performance (i.e., increased immobility) in the open field may derive of a disturbance of the functional architecture of dopaminergic networks, they are also compatible with the clear emotional disturbances observed after perinatal exposure to cannabinoids (for reviews, see Arevalo et al., 2001; Navarro et al., 1995; Rubio et al., 1998) or that described in cannabinoid CB1 receptor knockout mice (Martin et al., 2002). Additionally, the present study shows that, as described, the effects on female rats were less intense, supporting the existence of a sexual dimorphism on the developmental effects of cannabinoids (for reviews, see Fernandez-Ruiz et al., 1994; Navarro et al., 1995).

In the present study, a challenge with dopaminergic agonists, in the adult period, on rats perinatally exposed to Δ^9-THC, revealed the existence of permanent subtle alterations in the regulation of dopaminergic transmission. Perinatal Δ^9-THC exposure accentuated the marked inhibition of locomotion and the increased immobility induced by the administration of a low dose of apomorphine, and the initial inhibitory motor depressant phase evoked by quinpirole. Since the early component of motor inhibition induced by dopaminergic agonists has been considered a robust index of autoreceptor activation (Yarbrough et al., 1984), these effects might reflect the existence of alterations of dopamine D_2-like autoreceptors induced by the perinatal exposure to Δ^9-THC (Fernandez-Ruiz et al., 1994; Rodriguez de Fonseca et al., 1991).

The observed effects (a significant increase in immobility and a marked decrease in locomotion after apomorphine or shortly after the injection of quinpirole) could be due to a decrease in dopamine outflow derived from the enhanced responses of dopamine autoreceptors controlling dopamine synthesis and release. The administration of low doses of quinpirole produce a biphasic motor effects characterised by transient suppression of movement, which may be mediated by dopamine D_2 family autoreceptors, followed by a longer lasting hyperactivity, that includes increased stereotyped behaviours, due to activation of postsynaptic dopamine D_2 family receptors (Eilam and Szechtman, 1989; Frantz and Van Hartesveldt, 1995; Picetti et al., 1997). Perinatal pretreatment with Δ^9-THC significantly enhanced the initial phase of increased immobility (at time of 5 min), whereas it reduced the subsequent phase of motor stimulation, as shown in the dose-dependent decrease in stereotyped activity in the Δ^9-THC perinatally exposed groups (at time of 60 and 120 min) after the acute administration of quinpirole (Fig. 6).

Functional interactions between the endogenous cannabinoid system and the dopamine D_2 receptor have been reported by previous studies in rats and primates, revealing the existence of a modulatory role for the endocannabinoid system on dopaminergic signalling (Giuffrida et al., 1999; Beltramo et al., 2000; Di Marzo et al., 2000a; Meschler et al., 2000). The cannabinoid CB_1 receptors are densely expressed on intrinsic striatal cells expressing dopamine receptors, a key component that controls execution of motor behaviours (Herkenham et al., 1990, 1991). Although the nature of the modulatory role for the endogenous cannabinoid system on dopamine-mediated behaviours has not been fully understood (Giuffrida et al., 1999; Di Marzo et al., 2000b; Gubellini et al., 2002), our data suggest that perinatal exposure to THC might disrupt normal dopaminergic signalling in motor brain areas, probably by affecting permanently the intimate relationships between dopamine receptors and endocannabinoid system. That alteration seems to persist until adult age showed by an increased sensitivity to the effects of a challenge with dopaminergic agonists. Further studies are required to identify whether these alterations reflect changes in dopamine receptor-mediated signalling (Rodriguez de Fonseca et al., 1991) or in cannabinoid CB_1 receptors or endocannabinoid production (for a review, see Fernandez-Ruiz et al., 2000).

Despite this, many of the cannabinoid receptors are located presynaptically on neurons where their activation causes the inhibition of the respective transmitter (Schlicker and Kathmann, 2001). Therefore, the role of endocannabinoids as modulators of the neurotransmitter release (Di Marzo et al., 1998) has a special relevance, because the perinatal treatment with Δ^9-THC could alter this regulatory role, modifying the modulation of dopamine and GABA neurotransmitters in the basal ganglia, and that might explain the inhibition of motor activity. Although cannabinoid CB_1 receptors are not expressed in dopamine terminals in adult animals, they are present in developing monoaminergic neurons expressing tyrosine hydroxilase (Hernandez et al., 2000). Continuous stimulation of cannabinoid CB_1 receptors during development might result in alteration of presynaptic dopamine D_2 receptor function, revealed only after pharmacological challenges in adult animals. The differential effects of perinatal cannabinoids on presynaptic and postsynaptic dopamine D_2 receptors might be explained on the different nature of both kinds of receptors, as suggested

recently by studies using targeted mutations of the two isoforms described: the dopamine D_2 short and the dopamine D_2 long receptors (Usiello et al., 2000).

In summary, the results observed in the present study are consistent with previously reported findings which reflect that early life experiences such as prenatal stress or drug abuse constitutes vulnerability factors that could produce long term effects. The administration of low doses of THC during the perinatal period constitutes a vulnerability factor for developmental alterations in the dopaminergic system in the brain, due to the modulatory role of the endogenous cannabinoid system along brain development. These changes in the sensitivity of dopamine receptors caused by the administration of Δ^9-THC during the perinatal period may have implications for our understanding of neuropsychiatric disorders associated with neurodevelopmental alterations such as schizophrenia or drug addiction.

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