The Spanish Journal of Psychology
1998, Vol. 1, No. 1, 39-58

1138-7416

Early Cannabinoid Exposure as a Source of Vulnerability to Opiate Addiction: A Model in Laboratory Rodents

Miguel Navarro* and Fernando Rodríguez de Fonseca
Complutense University of Madrid

Recent findings have identified an endogenous brain system mediating the actions of *cannabis sativa* preparations. This system includes the brain cannabinoid receptor (CB-1) and its endogenous ligands anandamide and 2-arachidonoyl-glycerol. The endogenous cannabinoid system is not only present in the adult brain, but is also active at early stages of brain development. Studies developed at our laboratory have revealed that maternal exposure to psychoactive cannabinoid results in neuro-developmental alterations. A model is proposed in which early Δ^9-tetrahydrocannabinol (THC) exposure during critical developmental periods results in permanent alterations in brain function by either the stimulation of CB-1 receptors present during the development, or by the alterations in maternal glucocorticoid secretion. Those alterations will be revealed in adulthood after challenges either with drugs (i.e. opiates) or with environmental stressors (i.e. novelty). They will include a modified pattern of neuro-chemical, endocrine, and behavioral responses that might lead ultimately to inadaptation and vulnerability to opiate abuse.
Key words: rat, cannabinoids, perinatal exposure, behavior, opiates, conditioning, ACTH, corticosterone, vulnerability.

En los ultimos años se ha logrado identificar un sistema endógeno que media las acciones de los principios activos del cannabis sativa. Este sistema está compuesto por el receptor para cannabinoides cerebral (CB-1), y sus ligandos endógenos, la anandamida y el 2-araquidonilglicerol. El sistema cannabinoide endógeno está activo en el cerebro adulto, participando también en el desarrollo cerebral. Los estudios desarrollados en nuestro laboratorio han demostrado que la exposición maternal durante la gestación y la lactancia a compuestos activos en el receptor CB-1 conducen a alteraciones en el desarrollo cerebral. En este trabajo se propone un modelo en el cual la exposición durante periodos críticos del desarrollo a Δ^9-tetrahydrocannabinol (THC), el principal cannabinoide psicoactivo del *cannabis sativa*, induce cambios permanentes en la función cerebral, bien mediante la activación de los receptores CB-1 presentes durante edades tempranas del desarrollo, o bien mediante la alteración de la secreción maternal de glucocorticoides. Estas alteraciones se manifiestan en la edad adulta tras la exposición a desafíos adaptativos, como el tratamiento con drogas de abuso (opiáceos) o el estrés medioambiental (novedad, etc.), que inducen respuestas neuroquímicas,hormonales y comportamentales anómalas, que se manifiestan como una mayor vulnerabilidad a la adicción.
Palabras clave: rata, cannabinoides, exposicion perinatal, conducta, condicionamiento, morfina, glucocorticoides.

This work was funded by the Comisión Interministerial de Ciencia y Tecnología (CICYT, grant PM 96/0047), the Universidad Complutense de Madrid (Proyecto Multidisciplinar PR218/94-5670) and the Delegación del Gobierno para el Plan Nacional Sobre Drogas.

The authors wish to acknowledge the hard work and expert assistance of Pilar Rubio, José Luis Martín-Calderón, Ignacio del Arco, Raquel Gómez, Olivia Maestre, Leticia Escuredo, Yolanda Martín and Cristina Meseguer; and they also thank José Flórez Uría for his technical support. They also thank Dr. María A. Villanúa and Dr. Raúl M. Muñoz for their generous support in hormonal determinations. The authors are indebted to the National Institute on Drug Abuse (NIDA, USA) for providing Δ^9-THC, and to Raphael Mechoulam for the gift of HU-210.

* Correspondence concerning this article should be addressed to Dr. Miguel Navarro, Departamento de Psicobiología. Facultad de Psicología. Universidad Complutense de Madrid. 28223 - Madrid (Spain). E-mail: pspsc10@sis.ucm.es

The use of *Cannabis sativa* preparations (hashish, marijuana) has a long history, from folkloric use in ancient times, to the wide-spread recreational use in the twentieth century (Mechoulam, 1986). However, it is only within the past 30 years that researchers have begun to understand the biological basis of the pharmacological effects of *cannabis*. Moreover, the last 10 years have been crucial for identifying the neural substrates underlying cannabis actions in the brain (see Table 1, section A). Since the seminal discovery of the cannabinoids, the psychoactive compounds of cannabis, with the isolation of Δ^9-tetrahydrocannabinol (THC) as the main cannabinoid (Gaoni & Mechoulam, 1964), extraordinary advances have led to the synthesis of psychoactive cannabimimetic agents (Dewey, 1986; Mechoulam et al., 1987), and to the identification of an endogenous system mediating the neuro-pharmacological actions of cannabinoids. This endogenous system, the *endocannabinoid system*, is composed of the CB-1 brain cannabinoid receptor (Devane, Dysarz, Johnson, Melvin, & Howlett, 1988; Herkenham et al., 1990; Matsuda, Lolait, Brownstein, Young, & Bonner, 1990), and several brain constituents that have been proposed as endogenous ligands for the CB-1 receptor, including anandamide (Devane et al., 1992; Di Marzo et al., 1994) and 2-arachydonoylglycerol (Mechoulam et al., 1995; Stella et al., 1997). Surprisingly, the CB-1 cannabinoid receptor is present in brain areas, including those relevant for the rewarding properties of drugs of abuse (Gardner & Lowinson, 1991), in greater abundance than most other known receptors for neurotransmitters (Herkenham et al., 1990).

Since the isolation of THC, many studies have addressed the effects of this and other active cannabinoids in brain function (Dewey, 1986). The pharmacological profile of the cannabinoids includes the acute induction of analgesia, hypothermia, immobility, and catalepsy (Abood & Martin, 1992; Martín-Calderón et al, 1998), as well as neuro-endocrine alterations, including the activation of the pituitary adrenal axis and the inhibition of the secretion of most anterior pituitary hormones (Martín-Calderón et al., 1998; Rodríguez de Fonseca et al., 1991b, Rodríguez de Fonseca, Villanúa, Muñoz, San-Martín-Clarke, & Navarro, 1995b). Cannabinoids also activate monoaminergic systems in the brain, especially reward-related systems (Gardner & Lowinson, 1991; Molina-Holgado, F., Molina-Holgado, E., Leret, González, & Reader, 1993; Navarro et al., 1993a, 1993b, in press; Rodríguez de

Table 1

A. Major Advances in Cannabis Research. B. Contributions of the Research Trends developed since 1993 at the Instituto Universitario de Drogodependencias, Departamento de Psicobiología, Facultad de Psicología, Universidad Complutense de Madrid.

EVENT	REFERENCE
A.	
MEDICAL USES OF CANNABIS	3000 B.C. Traditional Asiatic Medicine. Mechoulam et al., 1986
ISOLATION OF PSYCHOACTIVE CANNABINOIDS	Gaoni and Mechoulam, 1964
SYNTHETIC CANNABINOIDS	Mechoulam et al., 1987
DISCOVERY OF CANNABINOID RECEPTORS (CB-1)	Devane et al., 1988; Herkenham et al., 1990
CLONING OF CB-1	Matsuda et al., 1990
ISOLATION, BIOCHEMISTRY, & ROLES OF ANANDAMIDE	Devane et al., 1992; Fride and Mechoulam, 1993; Di Marzo et al., 1994
SYNTHESIS OF CB-1 ANTAGONIST	Rinaldi-Carmona et al., 1994
ISOLATION & ROLES OF 2-ARA-G	Mechoulam et al., 1995; Stella et al., 1997
DESCRIPTION OF UPTAKE SYSTEMS	Beltramo et al., 1997
B.	
BEHAVIORAL TERATOLOGY OF CANNABINOIDS	Navarro et al., 1994ab, 1995, 1996; Rubio et al., 1995, 1998
CANNABINOIDS & DOPAMINERGIC SYSTEMS	Navarro et al. 1998; Rodriguez de Fonseca et al., 1994a and b
NEUROENDOCRINOLOGY OF CANNABINOIDS	Martín-Calderón et al., 1998; Rodríguez de Fonseca et al., 1995
CANNABINOIDS, STRESS, & ANXIETY	Navarro et al., 1997; Rodríguez de Fonseca et al., 1996
NEUROBIOLOGY OF CANNABINOID ADDICTION/DEPENDENCE	Rodríguez de Fonseca et al., 1997

Fonseca et al., 1992b; Rodríguez de Fonseca, Martín-Calderón, Mechoulam, & Navarro, 1994a), and induce anxiety-like behaviors in laboratory animals and humans (Dewey, Gardner & Lowinson, 1991; Halikas, Weller, Mouse, & Hoffman, 1985; Navarro et al., 1993a; Rodríguez de Fonseca et al., 1996). Most of these effects can be elicited after acute administration of anandamide (Fernández-Ruiz et al., 1997; Fride & Mechoulam, 1993; Romero et al., 1996) although with some exceptions (Fride et al., 1995). Additionally, the recent synthesis of the first cannabinoid receptor antagonist, SR 141716A (Rinaldi-Carmona et al., 1994) has contributed to establish the existence of an endogenous cannabinoid tone, regulating both motor activity and emotional responses (Gueudet, Santucci, Rinaldi-Carmona, Soubrié, & Le Fur, 1995; Navarro et al., 1997) and has provided a tool with which to unmask the neuro-adaptations underlying chronic cannabis exposure (Rodríguez de Fonseca , Carrera, Navarro, Koob, & Weiss, 1997).

The investigation of the effects of maternal exposure to cannabinoids on brain development has been a very active field of research in the cannabinoid field. Animal research has revealed the existence of long-term behavioral consequences of maternal exposure to drugs of abuse during gestation and lactation (Navarro,Rodríguez de Fonseca, Hernández, Ramos, & Fernández-Ruiz, 1994a; Navarro, Rubio, & Rodríguez de Fonseca, 1994b; Robins & Mills, 1993; Zuckerman, 1991). However, knowledge of the real impact of maternal exposure to cannabis on the development and adult expression of cognitive and behavioral functions in humans is far from being achieved. Cannabis sativa preparations remain the most widely used illicit drugs during pregnancy in western countries (Abel, 1980; Day et al., 1994; Fried, 1995). Cannabinoids can be transferred from the mother to the offspring through placental blood during gestation (Hutchings, Martin, Gamagaris, Miller, & Fico, 1989) and through maternal milk during lactation (Jakubovic, Hattori, & Mc Geer, 1977). The presence of psychoactive cannabinoids in the developing brain might interfere as epigenetic factors with the rigidly ordered developmental program that occurs during the ontogeny of the central nervous system, resulting in neuro-developmental alterations (Mirmiran & Swaab, 1987). Early laboratory studies had shown that maternal exposure to either cannabis extracts or THC resulted in long-lasting alterations in brain development and function (Abel; Brake, Hutchings, Morgan, Lasalle, & Shi, 1987; Dalterio & Bartke, 1979; Fernández-Ruiz, Rodríguez de Fonseca, Navarro, & Ramos, 1992; Hutchings, Morgan, Brake, Shi, & Lasalle, 1987; Rodríguez de Fonseca, Cebeira, Fernández-Ruiz, Navarro, & Ramos, 1991a; Rodríguez de Fonseca, Cebeira, Hernández, Ramos, & Fernández-Ruiz, 1990; Rodríguez de Fonseca, Hernández, de Miguel, Fernández-Ruiz, & Ramos, 1992a; Walters & Carr, 1986). Subsequent studies developed in our laboratory (Table 1, references in section B) revealed that maternal exposure to cannabinoids resulted in behavioral alterations which could be observed either during development or at adult ages. These alterations were associated with neurochemical disturbances in monoaminergic and neuro-peptide systems (Bonnin et al., 1994; Molina-Holgado et al., 1993; Rubio et al., 1995).

An unexplored aspect of the behavioral teratology of abused drugs is their possible role as a vulnerability factor for drug-seeking behavior in the adult. Several studies have shown that maternal exposure to opiates (Gagin, Kook, Cohen, & Savit, 1997) or to psychostimulants (Keller, Lefevre, Raucci, Carlson, & Glick, 1996) resulted in a sensitization to the reinforcing effects of these compounds in the adult offspring, suggesting the establishment of a vulnerability as a result of that early exposure. The neurobiological mediators for the establishment of such vulnerability remains to be determined. However, the pioneer work of McEwen and colleagues (1987, and references therein) has established that underlying such alterations may be the drug exposure-induced effects on maternal steroid-hormone secretions or the intrinsic activity of such drugs, which act as steroid hormone-like substances. Most drugs of abuse are potent activators of the hypothalamo-pituitary-adrenal axis (HPA), and it has been proposed that maternal stress induced by these drugs might underlie the observed behavioral sensitization (Molina, Wagner, & Spear, 1994; Rubio et al., 1995). The effects of such epigenetic influences in brain reward systems have been studied by the extensive research of Piazza's group (Piazza, Deminiere, Le Moal, & Simon, 1989; Piazza, Deroche, Deminiére, Maccari, Le Moal, & Simon, 1993; Piazza & Le Moal, 1996). These authors have suggested that both prenatal stress and prenatal drug exposure seem to play an important role in the individual predisposition to psychostimulant self-administration in rodents, by the induction of long-term changes in the activity of mesocorticolimbic- projecting dopamine neurons containing glucocorticoid receptors (Harfstrand et al., 1986), and HPA activity in the adult offspring (Callaghan et al., 1994; Deminiére et al., 1992; Maccari et al., 1991). In this regard, there is little information about the possible long-term vulnerability-inducing effects of maternal exposure to marijuana or its psychoactive compounds. Natural cannabinoids, such as THC are potent activators of the HPA axis by means of its interaction with hypothalamic brain cannabinoid receptors (Rodríguez de Fonseca et al., 1991b,1995b). Perinatal exposure to cannabinoids might then result in increased maternal circulating levels of corticosterone and could interfere with the development of the HPA axis in the fetuses. The fact that brain cannabinoid receptors are present in early stages of development (Rodríguez de Fonseca, Ramos, Bonnin, & Fernández-Ruiz, 1993) adds another important biological substrate to the actions of maternally-delivered cannabinoids on brain development.

The present study was designed to further analyze the effects of maternal exposure to THC in the reinforcing properties of morphine in the adult offspring. Previous studies have shown that endogenous opioid systems (peptides and their receptors) can be altered after maternal exposure either

to stress (Insel, Kinsley, Mann, & Rridges, 1990; Keshet & Weinstock, 1995) or to perinatal cannabinoid treatments (Kumar et al., 1990). Because of the growing evidence that the functional status of the HPA-axis might be relevant for opiate-seeking behavior in the rat (Saham & Stewart, 1995), sensitivity to the reinforcing properties of a moderate dose of morphine (350 µg/kg, which induces place-conditioning in only 50% of the control animals) was studied in adult offspring born of mothers exposed during gestation and lactation to several doses of THC that were closely related to human consumption. The functional status of the HPA axis was evaluated by measuring plasma levels of ACTH and corticosterone in basal conditions, and after the adaptive challenge of conditioned place-preference (CPP) testing. Another stress-related hormone, prolactin (PRL), was also controlled. Since increased sensitivity to the reinforcing effects of abused drugs has been associated with changes in the pattern of locomotor and exploratory activity (Piazza et al., 1989), we also evaluated the behavioral response to novel and familiar environments, using a battery of behavioral tests. Lastly, in order to further evaluate the establishment of long-lasting functional alterations as a result of maternal cannabinoid exposure, we studied the effects of maternal exposure to the highly potent cannabinoid, (-)-11-hydroxy-Δ^8-tetrahydrocannabinol-dimethylheptyl (HU-210), on the adult sensitivity of the HPA axis to an acute cannabinoid challenge.

Method

Subjects

Female virgin rats of the Wistar strain (> 8 weeks old; 200-250 g) were housed in a room with controlled photoperiod (lights on: 08:00-20:00) and temperature (23 ± 1°C). They had free access to standard food (Panlab, Barcelona) and water. Daily vaginal smears were taken between 10:00-12:00 h, and only those animals exhibiting three or more consistent 4-day cycles were used in this study. Females in the proestrus 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 Δ^9- tetrahydrocannabinol or HU-210 exposure studies. The day on which sperm plugs were found was designated the first day of gestation. After weaning the offspring, they were separated and housed, 4-5 animals of the same sex and treatment per cage. Thirty litters were used for the behavioral studies, distributed as follows: 8 vehicle, 4 THC 1 mg/kg, 4 THC 5 mg/kg, 3 THC 20 mg/kg, 4 HU-210 1 µg/kg, 4 HU-210 5 µg/kg, and 3 HU-210 25 µg/kg. For both CPP and defensive-withdrawal studies, 2-3 male offspring and 2-3 female offspring per litter were chosen randomly at adult age (> 70 days). Female rats were studied in the estrous phase of the cycle.

Cannulae implantation. For the time course of the endocrine actions of HU-210, a group of six animals per treatment was implanted under Equithesin anesthesia with indwelling atrial cannulae, inserted via the right external jugular vein. Cannulae were filled with heparinized saline (10 U/ml) to maintain patency. Animals were allowed at least 48 hours for recovery, prior to experimental procedures.

All the procedures were carried out according to the European Communities Council directive of 24 November, 1986, (86/609/EEC) regulating animal research.

Experiments

In the first experiment, we studied the performance on the elevated plus-maze of male and female offspring who had undergone the different perinatal treatments with THC during postnatal development. The animals were repeatedly exposed to the maze at 20, 30, 40, and 70 days of postnatal life.

In the second experiment, adult animals of both sexes (>70 days), born of mothers exposed to the different experimental treatments, were studied in the defensive-withdrawal test under novelty conditions (Rodríguez de Fonseca et al., 1996). One week after the testing procedure, they were divided into two groups. Rats of the first group were left undisturbed for two additional weeks. The animals were killed after habituating them to the handling procedure, and plasma samples were obtained (basal group). The second group was used to study the effects of perinatal exposure to THC on the reinforcing properties of a moderate dose of morphine (350 µg/kg, Rubio et al. 1995), using the CPP paradigm. Locomotor activity was measured during conditioning sessions. These animals were killed after the end of the 45-minute testing session of the CPP test, and plasma samples were collected (place-preference group).

In the third experiment, animals exposed to (-)-11-hydroxy-Δ^8-Tetrahydrocannabinol-dimethylheptyl (HU-210) were used for evaluating the time-course of the adrenal response to an acute cannabinoid challenge. Adult animals from the different groups (n = 6; > 70 days) bearing indwelling atrial cannulae were injected with HU-210 (20 µg/kg, iv), and plasma samples were obtained from the jugular vein -20, 0, 30, 60, and 120 minutes before and after the injection of the cannabinoid agonist.

Drugs and Treatments

Perinatal cannabinoid exposure. Δ^9-tetrahydrocannabinol (THC) of greater than 95% purity was provided in an ethanol solution by the National Institute on Drug Abuse (Project 4886-OB). (-)-11-hydroxy-Δ^8-tetrahydrocannabinol-dimethylheptyl (HU-210)HU-210 was a gift by Dr. Raphael Mechoulam, from The Hebrew University at Jerusalem. It was dissolved in 95% ethanol at a concentration of 10 mg/kg. Immediately before

use, the ethanol was evaporated and the residue was emulsified using sesame oil as the vehicle. Pregnant females received a daily single oral dose of THC (1, 5 or 20 mg/kg bodyweight, given between 10:00 and 12:00 a.m.), HU-210 (1,5 or 25 µg/kg) or vehicle in a volume of 0.1 ml. The treatment started in the fifth day of gestation and was maintained until the 24th day after birth, the day on which pups were weaned. The doses of THC chosen were an extrapolation from current estimates of moderate to heavy exposure to this compound in humans, and were corrected, considering the differences in route of administration and body surface area (Rosenkrantz, Sprague, Fleischman, & Braude, 1975). We have previously observed that this dosage resulted in plasma THC levels within the range of those reported causing behavioral and physiological effects in animal models (Navarro et al., 1993b; Rodríguez de Fonseca et al., 1991b). HU-210 doses were selected on the basis of its equipotency with THC (Rodríguez de Fonseca et al. 1995b, 1996). To assess the possible toxic effects of the treatment previously reported (Brake, Hutchings, Morgan, Lasalle, & Shi, 1987; Hutchings et al., 1987), several gestational and lactational parameters were controlled, as described elsewhere (Navarro et al., 1994b). Figure 1 depicts the lack of consistent effects of maternal exposure to THC on food- and water-intake during gestation and lactation. A similar profile was observed after HU-210 exposure.

Morphine treatment. Morphine hydrochloride was used for the CPP studies. It was supplied by Centro Nacional de Estupefacientes y Psicótropos, prepared daily using isotonic saline as vehicle, and injected intraperitoneally at the doses described in the Experiments Section, in a volume of 0.3 ml. None of the morphine doses tested induced physical dependence, as evaluated using naloxone (1 mg/kg) after the 3-day conditioning sessions (Rodríguez de Fonseca et al., 1995a).

Acute HU-210 exposure. HU-210-induced alterations in the secretion of corticosterone and prolactin were studied in animals bearing indwelling atrial cannulae. HU-210 was prepared in saline/propylene-glycol/Tween 80 vehicle (90:5:5 v/v), and injected intravenously at a dose of 20 µg/kg in a volume of 100 µl.

Behavioral Testing

Elevated plus-maze. The performance of the offspring in the elevated plus-maze was repeatedly evaluated at postnatal days 20,30,40, and 70, following previously described methods (Rubio et al. 1995). The results were expressed as 1) the absolute time spent in the exposed arms of the maze, and 2) the number of arm entries. All the behavioral studies took place during the morning time of the light cycle (09:30 - 13:00 hr)

Defensive withdrawal. Defensive-withdrawal test was conducted as previously described (Rodríguez de Fonseca et al., 1996). The apparatus consisted of an opaque open field (100 x 100 x 40 cm), the floor of which was marked with 20 x 20 cm squares. The field contained a cylindrical polyethylene chamber, measuring 17 cm deep and 10 cm in diameter. The chamber was open at one end and situated alongside the wall running lengthwise and 20 cm away from a corner of the open field. The open field was illuminated using a 500 W halogen ceiling light, which was regulated to yield 350 lux at the center of the open field. Testing was conducted only under novelty conditions, as described in the Experiments Section. The rats were placed inside the chamber, in the open field, and the following behaviors were scored by trained observers, who were blind to experimental conditions: latency in leaving the chamber (emergence latency), defined as placement of all four paws in the open field; the total time spent in the chamber; the mean time spent in the chamber (i.e., the total time spent in the chamber divided by the total number of entries); motor activity, defined as the total number of lines on the floor of the open field crossed outside the chamber (crossings), and the number of rearings performed outside the chamber. The test length was 15 min. After testing each animal, the apparatus was cleaned with a weak acid solution (1% acetic acid) to prevent olfactory cues from affecting the behavior of subsequently tested rats.

Conditioning. Morphine-induced place preference (CPP) studies were performed as previously described (Rodríguez de Fonseca et al., 1995a), using a three-arm apparatus similar to that described by Hand, Stinus, & Le Moal (1989). The apparatus consisted of three interconnected rectangular boxes, measuring 40 x 35 x 35 cm, situated at 120° angles from each other. In the middle, there was a triangular area with a smooth glass floor, from which any of the three compartments were accessible. Each compartment was equipped with a different set of sensory stimuli: compartment A was equipped with a sand floor, plain walls, and a small container with a drop of 10% acetic acid. Compartment B contained a removable soft plastic floor, walls painted with white dot circles (7.5 cm), and a small container with a drop of anise extract. Finally, compartment C had a cork floor, alternating white strips (5 cm wide) painted on the walls, and no odor (a container with distilled water). The apparatus was placed in a dimly illuminated (110 Lux) isolated room. Each compartment was equipped with eight photocells which allowed us to monitor the position of the animal, and to automatically register the time spent in each compartment. After testing each animal, the floors were changed and washed to avoid odor cues. Each CPP experiment consisted of a 5-day schedule, with three phases: preconditioning, conditioning and testing. Animals exhibiting strong unconditioned aversions (< 10% of the session) or preferences (> 60% of the session) for any compartment in the 45-minute preconditioning session were discarded for the conditioning procedures. Those two compartments to which the animals exhibited the most similar preference-time were randomly assigned for the conditioning procedure. This consisted of a 3-day schedule of double conditioning sessions. The first day involved a morning session

(9:00 - 13:00) in which animals received a single dose of morphine (a full dose-response (experiment 1(or 350 g/kg ip, in THC-exposed animals) and were immediately placed in one of the compartments. During this 30-min conditioning session, the animals were not allowed to explore the other compartments of the apparatus. In the evening session (16:00 - 19:00) the animals received a single intraperitoneal injection of saline solution, and were placed for 30 min in the other compartment chosen for conditioning. On the second day of conditioning, the rats received the saline injections in the morning session and the drug administration in the evening session. On the third day of conditioning, the same schedule was used as on the first one. On the basis of preliminary studies in our laboratory, we chose this schedule to avoid circadian variability (morning/evening). After three days of conditioning, the animals were again allowed to freely explore the three compartments, as in the preconditioning phase (testing session). The absolute time spent in each compartment was automatically registered and used for the evaluation of the CPP.

Locomotor activity. During the different conditioning sessions, the position of the animal in the compartment was automatically registered by means of the photocells system described above, and the total number of beam interruptions was used for the analysis of locomotor activity (Rodríguez de Fonseca et al. 1995a).

Sampling and Hormonal Determinations

Three hundred microliters of blood were withdrawn at -20, 0, 30, 60, and 120 minutes before and after the injection of HU-210 from the jugular vein with a heparinized syringe. Sixty minutes after obtaining the last sample, the animals were sacrificed by rapid decapitation using a guillotine. Animals were previously familiarized with this handling procedure. Trunk blood was collected in tubes containing 400 µl of 6% EDTA, and centrifuged at 2500 x g at 4°C. Plasma was stored, frozen at -20°C, until assayed for hormonal determinations. Plasma corticosterone levels were measured by a radioimmuno-assay system (RIA), using a specific antibody from Bio Clin (Cardiff, Wales). This RIA-system yields basal values of corticosterone of 175 ± 25 ng/ml in undisturbed adult male animals, and 500 ± 70 ng/ml in stressed animals (Rodríguez de Fonseca et al., 1996). The variability of the method was 15.3%, and the detection limit was 62 pg/ml. Plasma PRL levels were measured, as previously described (Rodríguez de Fonseca et al., 1995b), by a specific double-antibody RIA-system, using materials kindly provided by the National Hormone and Pituitary Program (NIH, Bethesda, MD, USA). Values are expressed in ng/ml of reference preparation rPRL-RP3. The intra-assay coefficient of variation was 3.3% and the sensitivity was 0.025 ng/ml. All samples were measured in the same assay to avoid interassay variations.

Statistical Analyses

Two levels of analysis were performed in the present study. Data of individual animals were assessed by multifactorial analysis of variance, as required. Following a significant F value, post hoc analyses (Newman-Keuls) were performed for assessing specific group comparisons. Litter analyses were performed following Holson and Pearce (1992) suggestions, in order to assess the presence of litter effects. To this end, ANOVAs were performed using the mean litter value observed as the unit for analysis. All calculations were performed using the BMDP statistical package.

Results

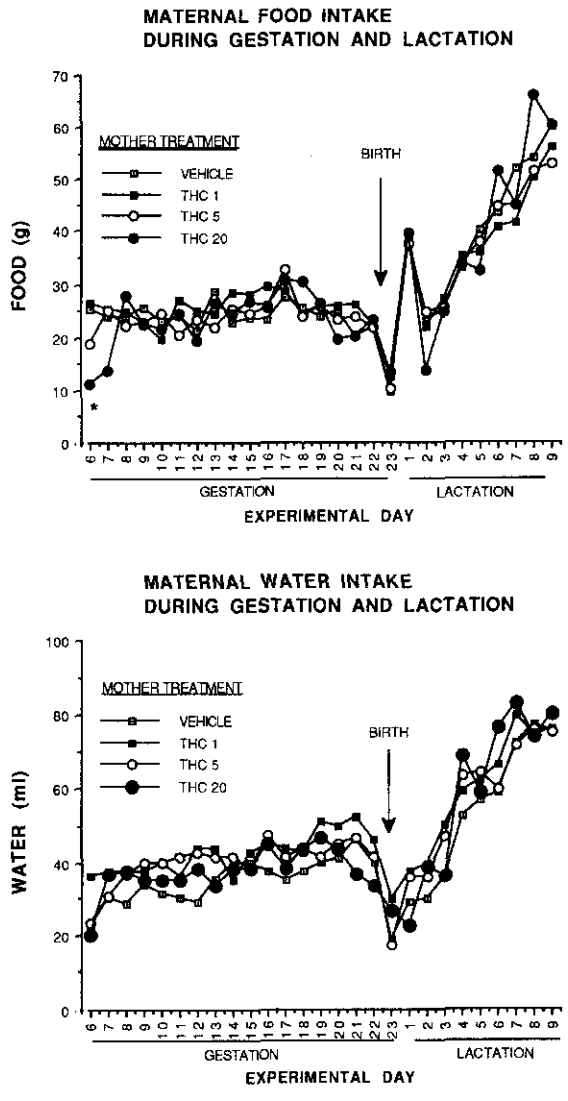

Figure 1. Lack of consistent effects of maternal exposure to THC (0, 1, 5 or 20 mg/kg) on food- and water-intake during gestation and lactation. Ordinates are means of the daily intake of either standard food pellets or tap water. (*) $p < 0.05$, Newman- Keuls, versus vehicle- treated animals.

Effects of Perinatal Exposure to THC on Several Gestational and Lactational Parameters

In order to assess the possible toxic and nutritional effects of THC administration, reported elsewhere (Brake et al., 1987; Hutchings et al., 1987), we recorded several parameters throughout the gestation and lactation (see Figure 1), as previously described (Navarro et al., 1994b). Our results showed that treatment with THC 20 mg/kg reduced maternal food-intake during the first day of treatment (simple effect of dose), $F(3, 12) = 7.4$, $p < .005$, and the second, $F(3, 12) = 8.7$, $p < .003$, disappearing thereafter. Moreover, overall ANOVA analysis did not reveal differences in maternal food-intake throughout the entire gestation, $F(3, 12) = 1.2$, $p = .35$ (nonsignificant). Mothers exposed to THC 1 mg/kg drank more water during gestation, $F(3, 12) = 4.6$, $p < .02$, although this effect disappeared during the lactational period. Perinatal THC exposure did not result in differences either in maternal weight-gain, or in the size and weight of the litters (data not shown). Weight gain of the offspring, measured on postnatal days 10, 15, 20, 30, and 40 was equal in all the experimental groups (data not shown).

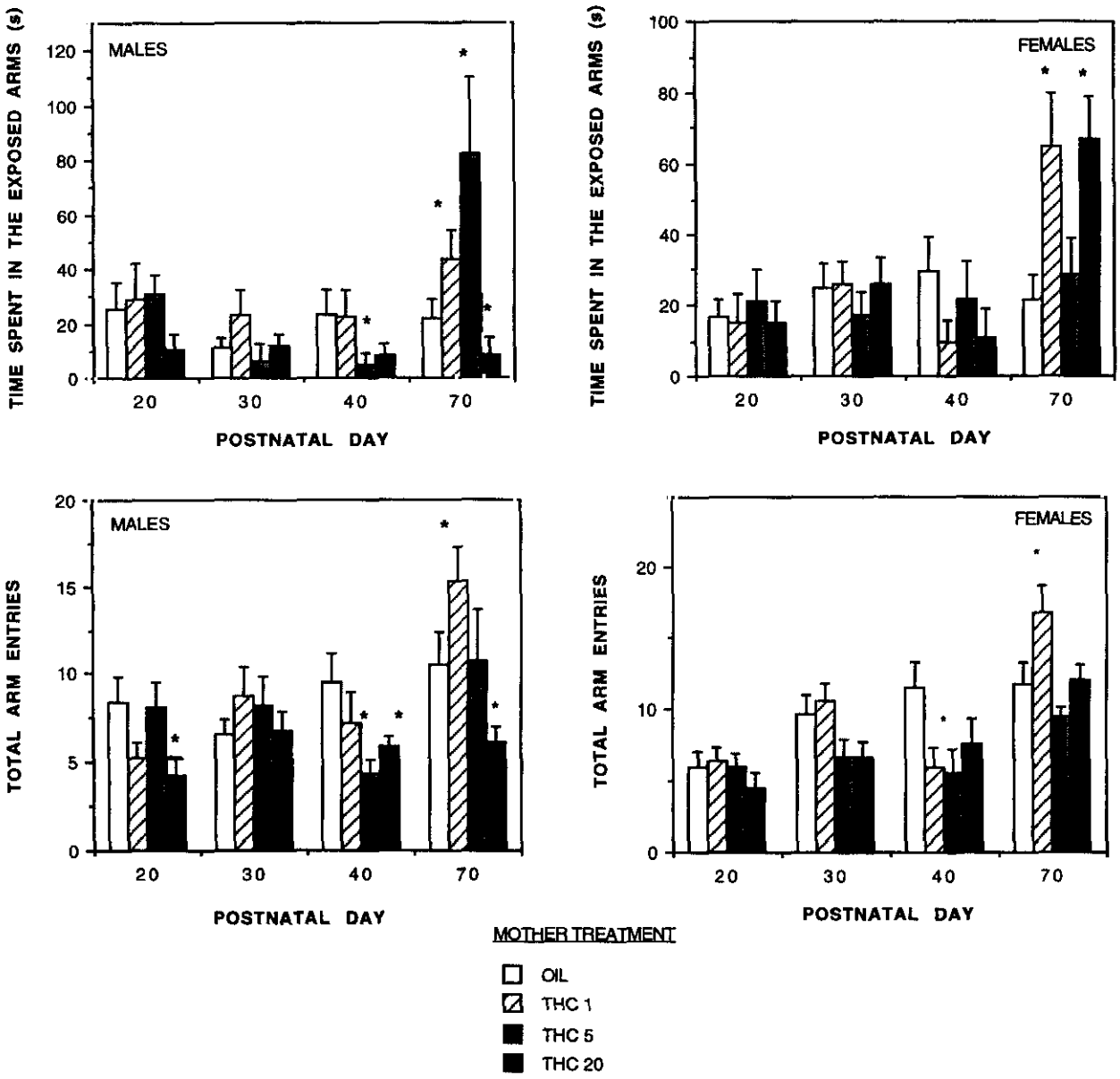

Figure 2. Developmental performance in the elevated plus-maze of male and female offspring perinatally exposed to THC (0, 1, 5 or 20 mg/kg). Upper panel, time spent in the exposed arms; lower panels, total arm entries. Values are means ± SEM. of 8-12 determinations per group. (*) $p < 0.05$, Newman- Keuls, versus vehicle-treated animals.

Effects of Maternal Exposure to THC on Behavioral Performance in the Elevated Plus-Maze

The analysis of the performance in the elevated plus-maze revealed a developmental effect both in the time spent in the exposed arms and in the number of arm entries, $F(3, 303) = 12.7$, $p < .0001$, and $F(3, 303) = 22.2$, $p < .0001$, respectively, with a markedly different response in the adult age (postnatal day 70). Both effects appeared as a result of maternal exposure to THC: main effect of maternal treatment, $F(9, 303) = 1.9$, $p < .05$; and $F(9, 303) = 3.6$, $p < .005$, for time spent out and arm entries respectively. However, the nature of the alterations in exploratory and locomotor behaviors seemed to be different. Thus, a clear sex x treatment x postnatal-day interaction appeared in the analysis of the time spent in the exposed arms of the maze, $F(9, 303) = 3.9$, $p < .01$: male offspring of mothers exposed to either THC 1 or 5 mg/kg exhibited higher exploratory behaviors, whereas those exposed to THC 20 mg/kg exhibited a constant tendency to avoid the exploration of the exposed arms of the maze. Female offspring also exhibited changes in the pattern of exploration of the open arms on postnatal day 70, although the changes were not dose-dependent, appearing at THC 1 and 20 mg/kg, but not at 5 mg/kg. Regarding locomotor activity in the maze, male offspring of mothers exposed to THC 20 mg/kg exhibited a significant decrease in the number of arm entries throughout development. Adult offspring exposed to THC 1 mg/kg exhibited an opposite pattern on postnatal day 70, with a high number of arm entries. Females displayed some changes throughout development, but they were not consistent, and were not present at adult ages.

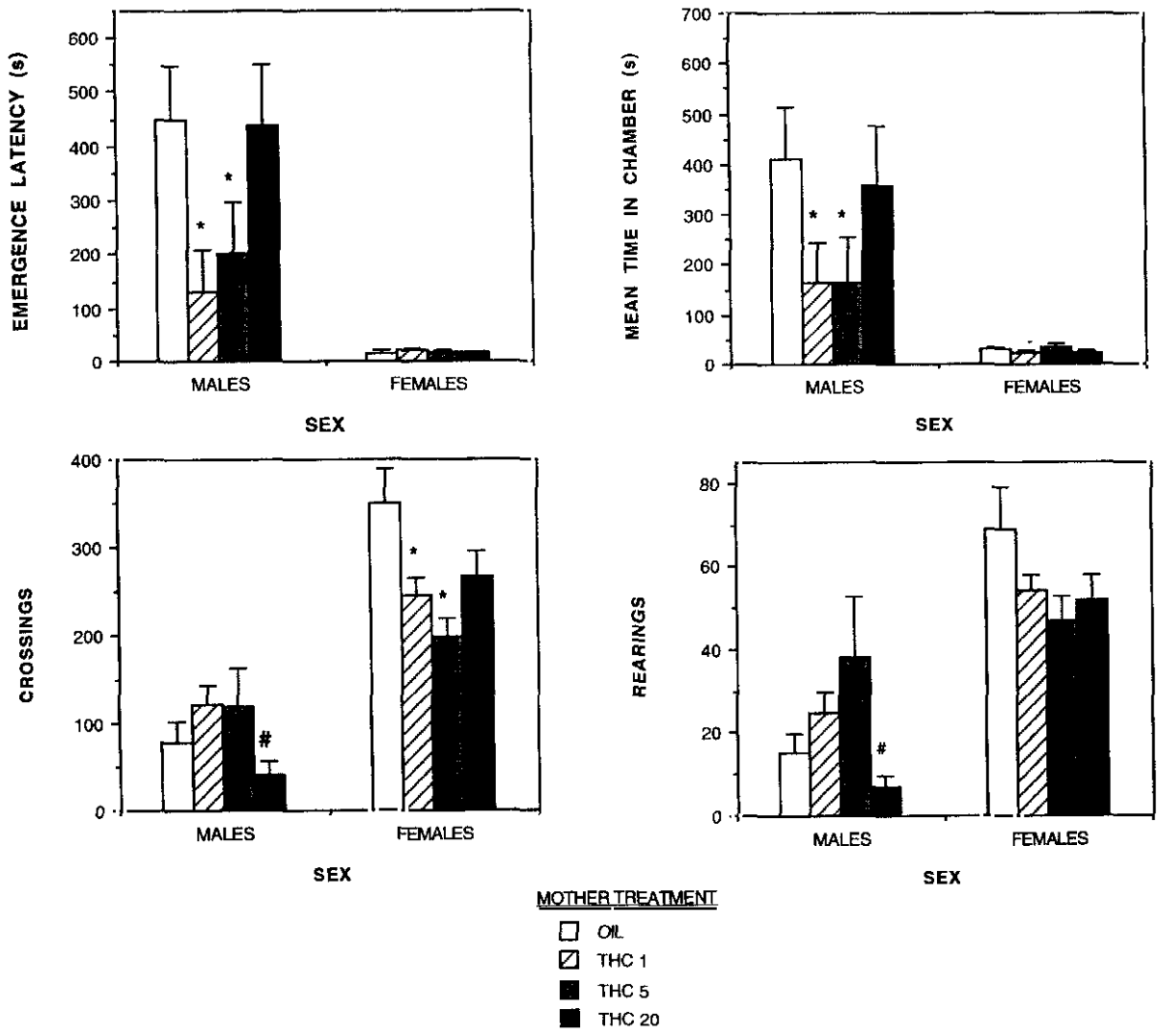

Figure 3. Behavioral reactivity to novelty, as measured in the defensive-withdrawal test, in male and female animals born of mothers exposed to THC (0, 1, 5 or 20 mg/kg). Ordinates are means ± SEM. of 8-12 determinations per group. (*) $p < 0.05$, Newman- Keuls, versus vehicle-treated animals.

Effects of Exposure to THC on the Performance of Adult Offspring in the Defensive-Withdrawal Test under Novelty Conditions

The pattern of behavior displayed in the defensive withdrawal test was clearly sex-dimorphic, as reflected in the several parameters scored: female animals emerged before males, $F(1, 81) = 23.9$, $p < .0001$, they remained less time inside the tube, $F(1, 81) = 29.7$, $p < .0001$, and they exhibited higher motor-activity scores than males for crossings, $F(1, 81) = 69.2$, $p < .0001$, and for rearings, $F(1, 81) = 39.7$, $p < .0001$. Maternal exposure to THC 1 or 5 mg/kg, but not to 20 mg/kg resulted in a decreased emergence latency in male offspring: sex x treatment interaction, $F(3, 81) = 3.01$, $p < .04$. Male offspring of the THC 1 mg/kg group remained less time inside the tube, $F(1, 25) = 8.78$, $p < .01$, and they also exhibited a lower mean time spent in the small chamber when compared to control animals, $F(1, 25) = 5.51$, $p < .03$ (data not shown). A significant sex x treatment interaction was also observed in both the number of crossings, $F(3, 81) = 4.72$, $p < .005$, and rearings, $F(3, 81) = 4.2$, $p < .01$, scored during the test.

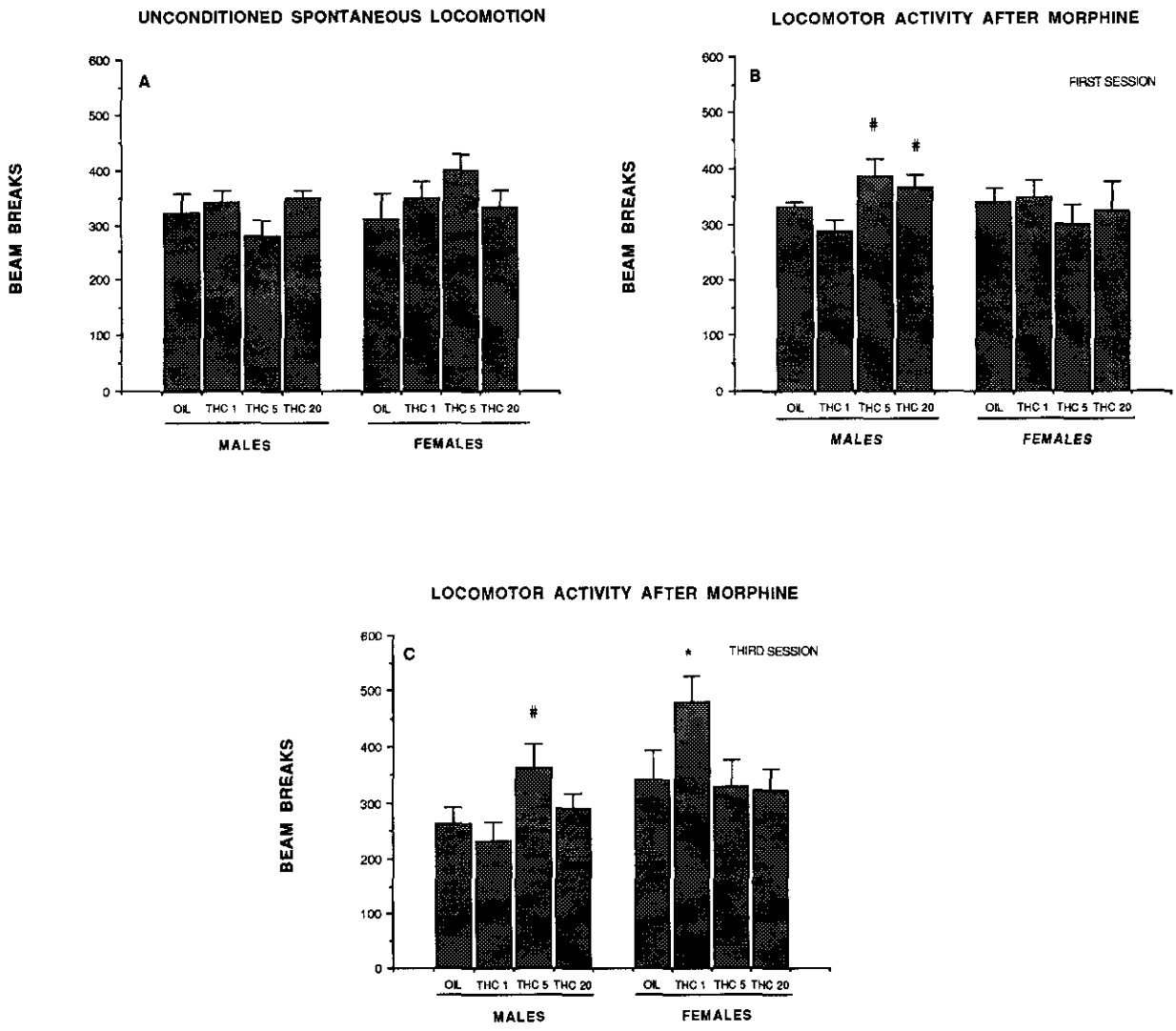

Figure 4. Locomotor activity of male and females animals perinatally exposed to THC (0, 1, 5 or 20 mg/kg) in the place-conditioning maze measured in: (A) untreated habituated animals; (B) during the first conditioning session with morphine; and (C) during the last conditioning session with morphine. Ordinates are the number of beam breaks registered for 45 min ± SEM of 8-12 determinations per group. (*) $p < 0.05$, Newman-Keuls, versus vehicle-treated animals.

Effects of Maternal Exposure to THC on Spontaneous and Morphine-Associated Locomotor Activity

Maternal exposure to THC did not result in alterations in spontaneous unconditioned locomotion exhibited by adult animals, independently of the sex variable. However, the response to morphine observed during conditioning sessions was sexually dimorphic, $F(1, 53) = 131.2$, $p < .005$, and was affected by perinatal exposure to THC, $F(15, 265) = 2.41$, $p < .05$. Animals of the vehicle group did not show altered locomotion after morphine 350μg/kg treatment during conditioning sessions (see Figure 4B and 4C). However, male animals born from mothers exposed to THC 5 or 20 mg/kg exhibited a differential response to first morphine when compared with THC 1 mg/kg group, $F(3, 53) = 3.7$, $p < .05$. This differential response could be still observed in the third conditioning session, on which a sensitized locomotor response to morphine appeared in animals of the THC 1 mg/kg group, $F(1, 12) = 4.1$, $p = .05$.

Effects of Maternal Exposure to THC on Morphine Place-Preference in Adult Offspring

Maternal exposure to low doses of THC resulted in enhanced sensitivity to the reinforcing properties of morphine 350 μg/kg displayed by the adult offspring, as measured in the place preference paradigm, $F(3, 124) = 4.4$, $p < .005$.The effect was sexually dimorphic, $F(1, 124) = 11.1$, $p < .001$. Male offspring of the THC 1 and 5 mg/kg dose group increased their preference for the morphine-paired compartment with respect to the saline-paired compartment, $F(3, 60) = 3.3$, $p < .03$. Only female offspring of the THC 1 mg/kg group increased their preference for the morphine-paired compartment with respect to the saline-paired compartment: simple effect of maternal exposure to THC 1 mg/kg, $F(1, 64) = 5.7$, $p < .03$. Neither control-offspring nor those born of mothers exposed to THC 20 mg/kg exhibited a clear preference for the morphine-paired compartment when compared to the saline-paired one.

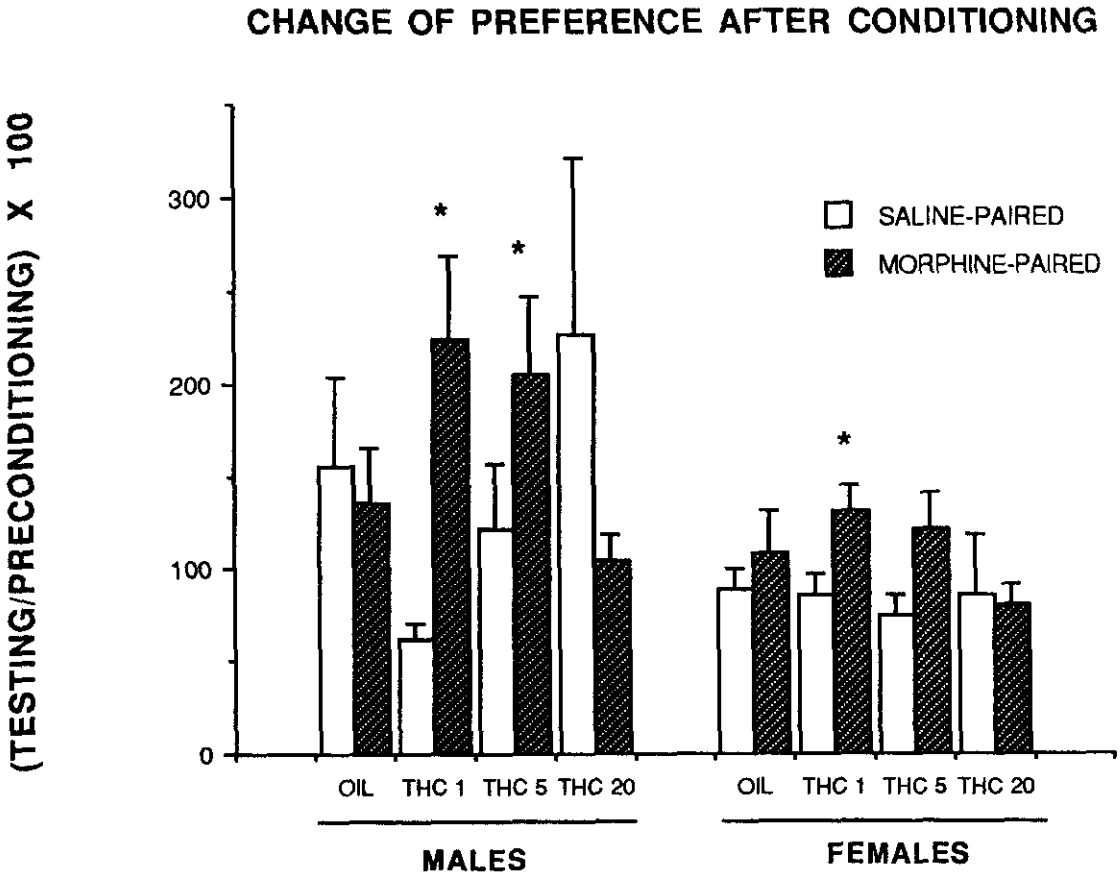

Figure 5. Morphine (350 μg/kg)-induced place conditioning expressed as the percentage of change over preconditioning time values. (*) $p < 0.05$, Newman-Keuls, morphine-paired versus saline-paired compartment.

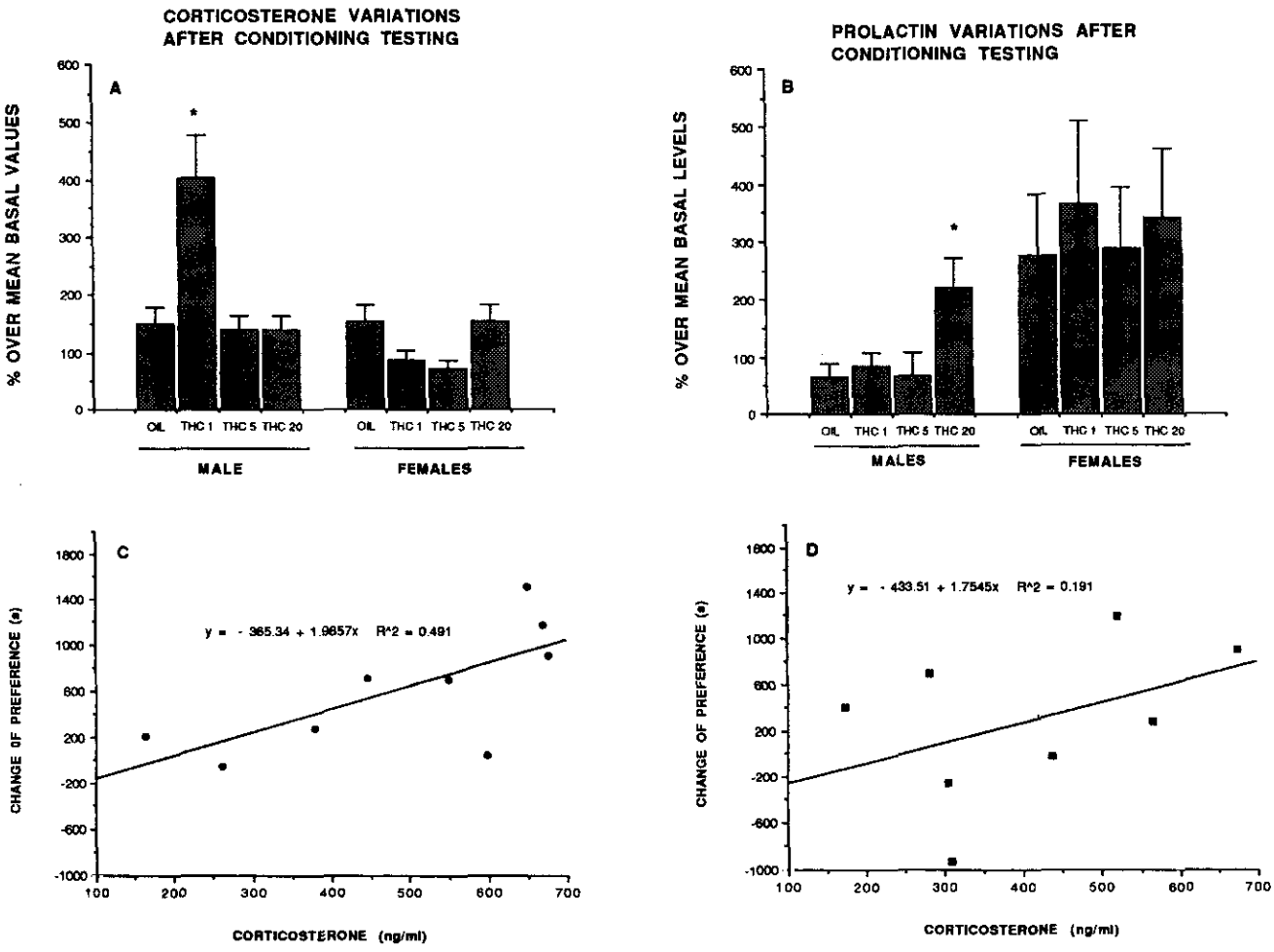

Figure 6. (A). Changes in corticosterone levels (exposed/basal ratio x 100) associated with the exposure to the conditioning maze in adult male and female animals born of mothers exposed to THC during gestation and lactation. (B) Changes in plasma-prolactin levels (exposed/basal ratio x 100) associated with the exposure to the conditioning maze in the same animals. (C) Positive correlation between plasma-corticosterone levels and preference for morphine-paired compartment in males of the THC 1 mg/kg dose group, but not in vehicle-group animals (D). (*) $p < 0.05$, Newman- Keuls, versus vehicle- treated animals.

Effects of Maternal Exposure to THC on Plasma Hormonal Levels after Morphine Place-Preference

Basal corticosterone levels (data not shown) were sex-dimorphic, $F(1, 83) = 12.03$, $p < .001$, and they were clearly affected by maternal exposure to THC: sex x treatment interaction, $F(1, 83) = 7.66$, $p < .01$. Maternal exposure to THC resulted in high basal corticosterone levels in female offspring and reduced basal levels of this steroid in male offspring of the THC 1 mg/kg group. Exposure to the place- preference test increased the levels of this adrenal steroid: test effect, $F(1, 141) = 10.2$, $p < .005$. The ratio, corticosterone levels (CPP groups)/average basal corticosterone levels, displayed marked sexual dimorphism, $F(1, 62) = 16.7$, $p < .0001$, showing a clear effect of maternal treatment, $F(3, 62) = 6.8$, $p < .0005$, and a marked sex x treatment interaction, $F(3, 62) = 12.85$, $p < .0001$, which revealed that male offspring of mothers exposed to THC-1 mg/kg displayed a higher increase in corticosterone levels as a result of the exposure to the CPP test (Figure 6). This activatory effect associated twith CPP testing was not observed in males from the low THC-exposure groups when plasma prolactin was analyzed. Moreover, it appeared in all females, independently of maternal THC exposure, and in males from the 20 mg/kg-dose group. Regression analysis revealed that the change of preference for the morphine-paired compartment correlates positively with plasma corticosterone levels in males of the THC 1 mg/kg-groups, $F(1, 8) = 6.75$, $p < .04$, $r = 0.49$. This positive correlation was not observed in the remaining experimental groups.

Table 2
Litter Analysis of the Effects of Perinatal Exposure to THC. Data are average litter Values ± SEM in basal and post-conditioned Place-Preference (CPP) Groups.

Parameters		Mother Treatment			
		Vehicle	THC 1 (mc/kg)	THC 5 (mc/kg)	THC 20 (mc/kg)
Emergence latency					
	Males	438.4 ± 152.8	87.9 ± 28.3 (*)	152.6 ± 36.5 (*)	298.7 ± 184.9
	Females	25.1 ± 8.2	26.9 ± 13.2	15.3 ± 5.1	14.6 ± 2.9
Change of preference					
	Males	284 ± 177.5	715.9 ± 246.2 (*)	386 ± 50.8	36.8 ± 84.4
	Females	-53.5 ± 73.6	192.5 ± 134.7	79.8 ± 22.9	-159 ± 79.1
Basal ACTH (pg/ml)					
	Males	126.1 ± 10	296 ± 73.7 (*)	130.4 ± 17.6	114.9 ± 42.4
	Females	109.9 ± 14.2	152.4 ± 31.6	169.7 ± 64.4	101.1 ± 4.9
ACTH post CPP (pg/ml)					
	Males	464.2 ± 34.3 (#)	248.6 ± 23.6 (*)	320.1 ± 46.5 (#)	292.3 ± 64.2 (#)
	Females	367.7 ± 93.3 (#)	220.9 ± 26.2	288.3 ± 73	218.0 ± 38.7 (#)
Basal Corticosterone (ng/ml)					
	Males	221.5 ± 63	123.4 ± 10 (*)	174.4 ± 13.3	271.9 ± 11.8
	Females	339.0 ± 73.3	646.1 ± 102.9 (*)	421.9 ± 111.8	394.2 ± 90.5
Corticosterone post CPP (ng/ml)					
	Males	408.5 ± 65.2	525.3 ± 77.9 (#)	384.0 ± 31.8	397.0 ± 84.1
	Females	413.7 ± 100.1	451.5 ±122.7	479.0 ± 110.5	529.5 ± 37.2

* $p < 0.05$, Newman Keuls versus vehicle-treated group. # $p < 0.05$, Newman Keuls versus basal group of the same treatment.

Litter Analysis of the Effects of Perinatal Exposure to THC

Litter analysis showed that perinatal exposure to THC 1 or 5 mg/kg resulted in a significant decrease in the emergence latency and in a tendency to exhibit a decrease in the total time spent in the compartment of the defensive-withdrawal test, $F(3, 22) = 3.91$, $p < .02$, and $F(3, 22) = 2.52$, $p < .08$, respectively. There was marked sexual dimorphism in the performance of this test, as described above, and perinatal THC exposure failed to induce alterations in female offspring. The effects of perinatal treatment with THC 1 mg/kg on morphine-induced change of place preference were present when the mean litter value was considered as the unit for analysis, $F(3, 19) = 3.8$, $p < .02$ (see Table 2). These effects were sexually dimorphic, $F(3, 19) = 8.7$, $p < .008$, and were significant in males exposed to THC 1 mg/kg. ACTH analysis revealed the presence of increased plasma-ACTH levels as a result of the CPP testing, $F(1, 44) = 35.7$, $p < .0001$. There was a significant test x treatment interaction, $F(3, 44) = 5.4$, $p < .004$, revealing a disrupting effect of maternal exposure to THC 1 mg/kg on the pattern of ACTH levels displayed by both the basal and place-preference groups. Litter analysis confirmed the sex-dimorphic effects of maternal THC exposure on basal corticosterone levels: sex x treatment interaction, $F(3, 22) = 4.3$, $p < .02$; and the different response to the CPP test, $F(3, 44) = 3.8$, $p < .02$, in males and females as a result of the exposure to THC.

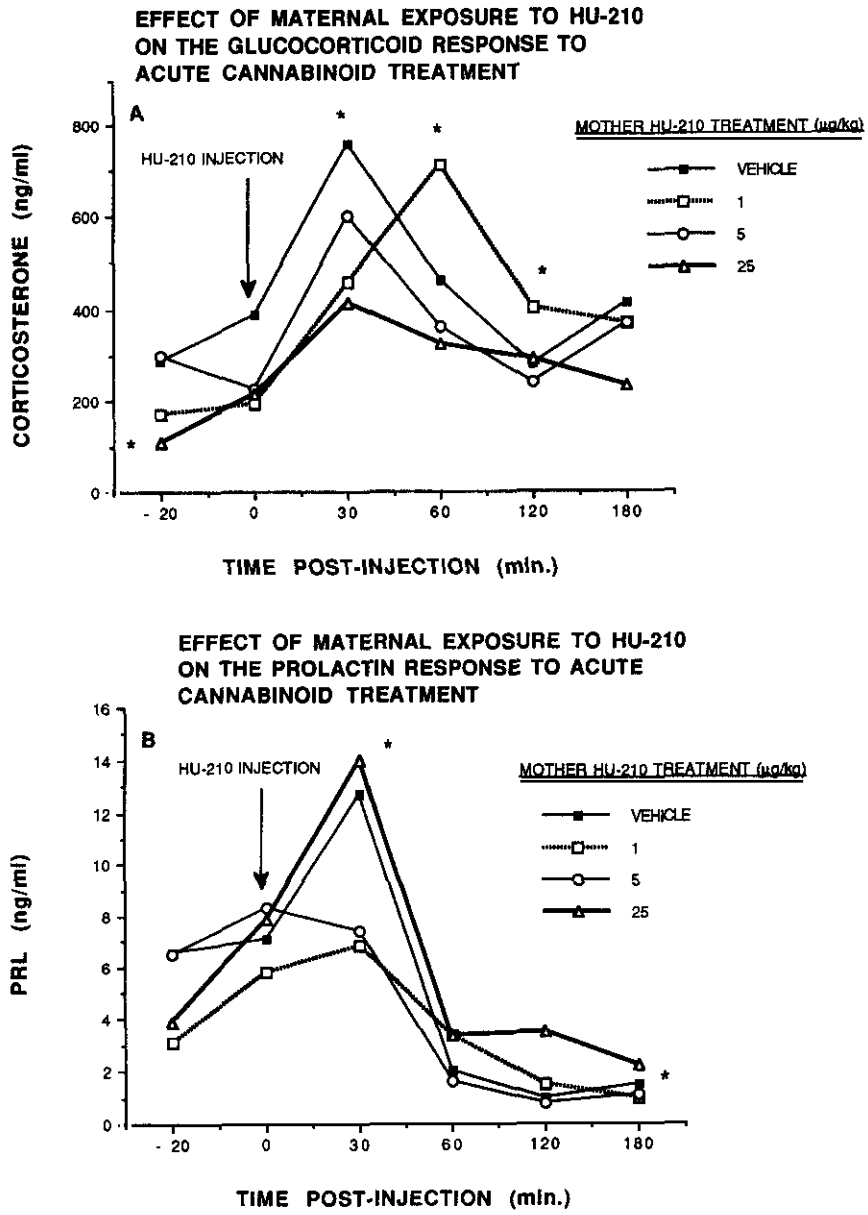

Figure 7. Maternal exposure to the cannabinoid receptor agonist HU-210 results in alterations in the response of both the pituitary-adrenal axis (A), and prolactin secretion (B), to an acute challenge with HU-210 in the adult offspring. (*) $p < 0.05$, Newman-Keuls, versus first fraction. Standard errors of the mean were omitted to improve the clarity of the graphs.

Effects of Maternal Exposure to HU-210 on HPA Sensitivity to an acute Cannabinoid Challenge

Maternal exposure to the highly potent cannabinoid receptor agonist HU-210 resulted in a permanently altered sensitivity of the HPA axis to the activating effects of cannabinoids: dose effect, $F(3, 16) = 3.7$, $p < .05$. Maternal exposure to HU-210 resulted in lower mean basal corticosterone levels (see Figure 7A). After acute injection of HU-210, the animals born from mothers of the 1 µg/kg-group exhibited a potentiated and long-lasting corticosterone response, with maximal increases up to 500% over mean basal levels: time effect $F(5, 80) = 6,1$ $p < .0001$. This effect was not observed in animals from the 25 µg/kg-dose group, which showed a more subdued response of shorter duration: time effect, $F(5, 80) = 1.2$, $p = .14$ (nonsignificant). Plasma-prolactin response to HU-210 in control animals was biphasic, with a short activating response observed 30 min after the injection and a subsequent long-lasting inhibition, $F(5, 80) = 15.07$ $p < .0001$ (see Figure 7B). Maternal exposure to HU-210 only affected the stimulatory phase of the response to HU-210, again in the lower-dose group: absence of simple effect of maternal treatment with HU-210, 1 µg/kg, on 30-min fraction, $F(5, 80) = 1.3$, $p = .25$ (nonsignificant).

Discussion

The present study shows that maternal exposure to the psychoactive constituent of cannabis THC results in a cluster of behavioral and endocrine alterations in the adult offspring that can be revealed after challenges with drugs (opiates, cannabinoids) or environmental modifications. Thus, maternal exposure to THC increased the sensitivity to the reinforcing properties of a moderate dose of morphine in the adult offspring, as measured in the CPP paradigm. This sensitivity was greater in animals exposed to low doses of this cannabinoid (1 and 5 mg/kg), and was found to be sexually dimorphic. Place-conditioning induced by morphine 350 µg/kg in male offspring perinatally exposed to THC 1 mg/kg was similar to that obtained in control animals using doses 3-6 times greater (Rodríguez de Fonseca et al., 1995a; Rubio, Rodríguez de Fonseca, Martín-Calderón, del Arco, Bartolomé, Villanúa, & Navarro, in press). A recent report indicates that this increased sensitivity is extensive to operant responses for opiates, using a model of morphine self-administration (Martin et al., 1996).The animals exposed perinatally to THC also exhibited sexually dimorphic changes in the behavioral reactivity to novel environments (see Figure 3 for the defensive-withdrawal study) and in the exploratory behaviors of a familiar environment with aversive properties (see Figure 2 for the plus-maze study). The endocrine alterations observed included sexually dimorphic alterations on the reactivity of the hypothalamo- pituitary-adrenal axis to the adaptive challenge of the CPP testing. Male offspring born of mothers exposed to THC (1 or 5 mg/kg) displayed normal to low basal levels of corticosterone, and an enhanced adrenal response to the CPP challenge. However, female offspring perinatally exposed to THC (1 or 5 mg/kg) displayed the opposite pattern: permanently elevated plasma levels of corticosterone and a blunted adrenal response to the HPA-activating effects of the CPP test. Lastly, males born of mothers exposed to the potent cannabinoid HU-210 exhibited a dose-related altered HPA response to an acute cannabinoid challenge: animals born from mothers exposed to the low dose of the cannabinoid (1 µg\kg) exhibited a sensitized and long-lasting response to acute HU-210 exposure, whereas those born of mothers treated with the higher dose (25 µg/kg) displayed a desensitized response to the acute cannabinoid challenge. Prolactin response displayed some subtle alterations in the lower-dose group, although they only affected the transient activatory effect of cannabinoid in its secretion. The results observed in males are consistent with previously reported findings which reflect that early life experiences, such as prenatal stress or perinatal exposure to opiates, amphetamine, and cocaine, might constitute a vulnerability factor for drug abuse (Callaghan et al., 1994; Deminiére et al., 1992; Gagin et al., 1997; Keller et al. 1996; Piazza & Le Moal, 1996).

An imbalance in the mesolimbic dopaminergic network has been considered as the neuro-chemical basis of the vulnerability to psychostimulant self-administration in rodents (Piazza et al., 1989). As described above, maternal exposure to cannabinoids not only results in developmental alterations of the dopaminergic systems in the brain (Bonnin et al., 1994; Rodríguez de Fonseca et al., 1990, 1991a, 1992a), but in permanent neuro-chemical alterations in adulthood (García et al. 1996; Navarro, de Miguel, Rodríguez de Fonseca, Ramos, & Fernández-Ruiz, 1996; Navarro, Rodríguez de Fonseca, Hernández, Ramos, & Fernández-Ruiz, 1994a; Navarro, Rubio, & Rodríguez de Fonseca, 1995; Rubio et al., 1995). If we consider these observations together with the data reflecting the alterations in the behavioral reactivity to novelty and the response to morphine, as well as the HPA alterations, we can argue that maternal exposure to THC leads to a vulnerable phenotype, partially resembling that proposed for amphetamine self-administration : mesocorticolimbic dopaminergic alterations, increased behavioral responses to novelty, and a sharp adrenal response to adaptive challenges (Piazza et al., 1989, 1993, 1996). This proposed model of vulnerability is depicted in Figure 8. Further research is needed to establish to what extent it can be also relevant for human species.

In the animals exposed to low doses of THC, the response to the CPP test was shifted to exhibit both an increased sensitivity to the reinforcing properties of an ED_{50} dose of morphine (resulting in a near 100% positive change of preference in perinatal-exposed animals), and a clear rise in plasma corticosterone levels, which were positively correlated. Interestingly, prolactin response to the CPP challenge was not correlated with the appearance of conditioning, although it was clearly affected by the exposure to THC 20 mg/kg.. The potent and long-lasting adrenal response to acute HU-210 exposure in animals born of mothers exposed to low doses of this cannabinoid is a remarkable finding, since this type of sensitized HPA response has also been proposed as a factor underlying vulnerability to psychostimulant self-administration (Maccari et al., 1991). Several mechanisms have been proposed in the elicitation of the perinatal effects of cannabinoids (for review, see Fernández-Ruiz et al. 1992 and Navarro et al., 1995). They include changes in opioid peptides and their receptors (Kumar et al., 1990), prenatal stress-like effects (Rubio et al., 1995), direct effects on developing monoaminergic systems (Bonnin et al., 1994; Navarro et al., 1996; Rodríguez de Fonseca et al., 1991a; Walters & Carr, 1986), and the activation of brain cannabinoid receptors which are present at birth (Rodríguez de Fonseca et al., 1993). Besides the previously described actions of cannabinoids on the developmental profile of dopaminergic cells, a possible cannabinoid-induced developmental alteration in opioid peptides and their receptors must be considered as a factor underlying the increased sensitivity to morphine-induced place preference. It has been previously described that perinatal cannabinoid exposure can (a) alter the developmental expression of opioid peptides in the rat brain (Kumar et al., 1990), and (b) induce changes in opioid-related

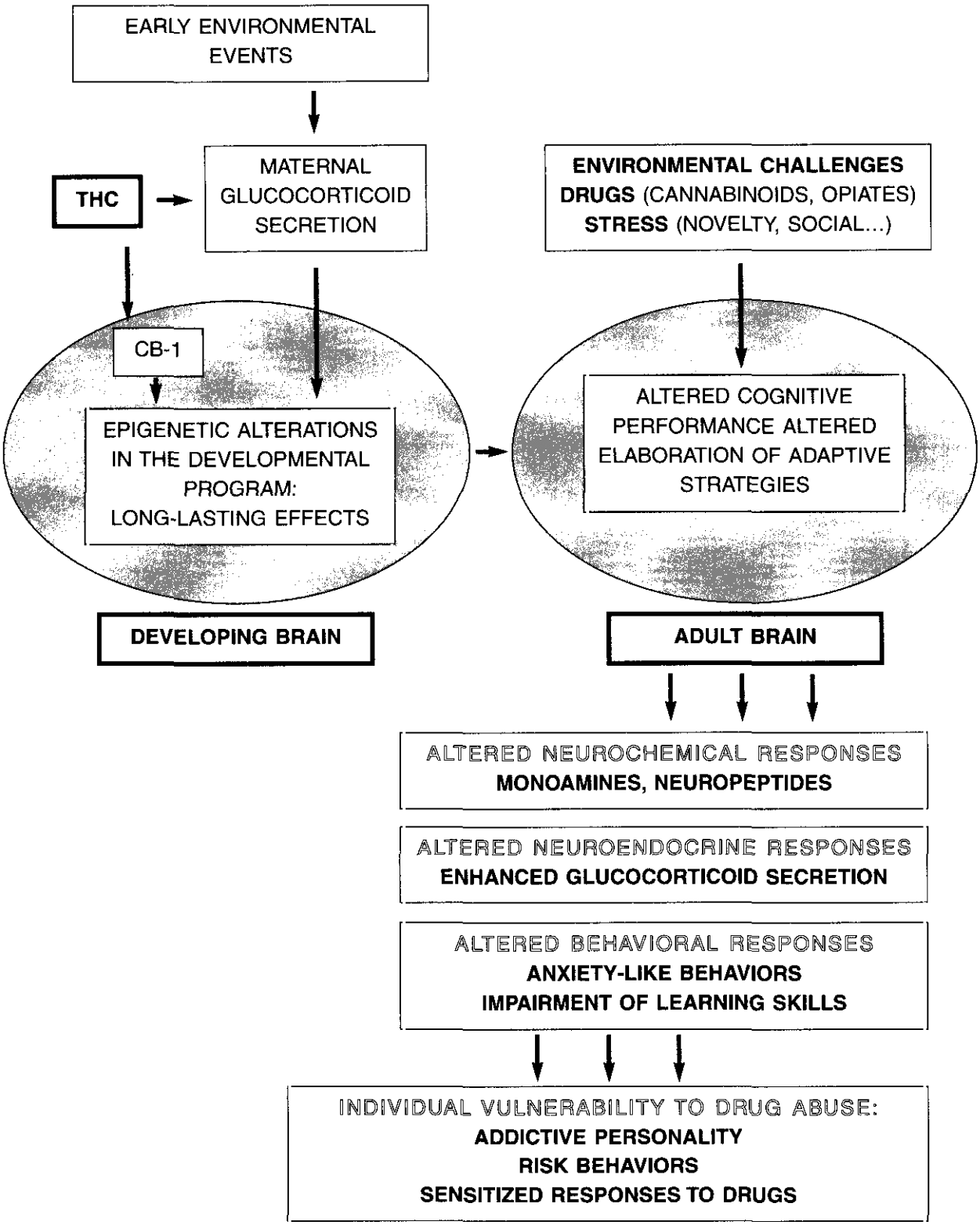

Figure 8. Proposed model of vulnerability to environmental challenges induced by maternal exposure to THC, the pyschoactive constituent of Cannabis sativa, in rodents. THC exposure during critical developmental periods may result in permanent alterations in brain structure/function by either the stimulation of CB-1 receptors present during the development, or the alterations in maternal glucocorticoid secretions, a known source of epigenetic-induced developmental plasticity. Adult challenges either with drugs (i.e. opiates) or with environmental stressors (i.e. novelty) will result in a modified pattern of neuro-chemical, endocrine, and behavioral responses, leading ultimately to inadaption and vulnerability.

behaviors (Vela, Fuentes, Bonnin, Fernández-Ruiz, & Ruiz-Gayo, 1995), namely a naloxone (5 mg/kg)-induced opioid-like abstinence syndrome in weanling males exposed to the cannabinoid, and developmental alterations in both pain sensitivity and the analgetic properties of morphine. Moreover, there is a clear association between endogenous opioid and cannabinoid systems in reward-related areas, supporting an important interaction in the modulation by the cannabinoid system of the reinforcing properties of opiates (Gardner & Lowinson, 1991).

The sexual dimorphism already reported, which has also been observed in the present study, is a common finding in perinatal cannabinoid studies (Dalterio and Bartke, 1979; Fernández-Ruiz et al., 1992; Navarro et al., 1994b), and might be reflecting the sex- dependent developmental profile of both opioid receptors (Hammer, 1985), and cannabinoid receptors (Rodríguez de Fonseca et al. 1993) in the rat brain. An additional explanation for the effects described after perinatal THC might be a possible cannabinoid-induced stress-like effect. This prenatal stress-like effect might occur through a THC-induced activation of maternal HPA axis, resulting in a rise of plasma-corticosterone levels in the fetuses, which are dependent on maternal levels (Ward & Weisz, 1984; see Figure 8). However, this possibility must still be conclusively determined. Recent studies (McCormick, Smythe, Sharma, & Meaney, 1995; Valleé et al., 1997) have described how maternal-restraint stress resulted in a permanent increase of basal corticosterone levels in the adult female offspring, which is the same finding that we have observed after perinatal exposure to THC, and which has also been found after perinatal exposure to alcohol in rats (Lee & Rivier, 1996; Taylor, Branch, Nelson, Fane, & Poland, 1986). Additionally, prenatal stress has been found to alter the opioid contribution to the behavioral response to novel environments (Poltyrev & Weinstock, 1997). In any case, it is worthy of note that the effects of perinatal THC on morphine sensitivity and HPA activity may be independent, although associated in male animals exposed to the lower dose of THC. It remains to be determined whether the association between morphine reinforcement and HPA activity is sexually dimorphic in naive animals, especially if we consider that the studies which have set a role for glucocorticoids on opiate reinforcement (see Saham & Stewart, 1995) have been performed only with males. Other gender-related findings arose from the present study: male rats born of mothers exposed to THC (1 or 5mg/kg) displayed more exploratory behavior in the defensive-withdrawal paradigm, which might be considered to resemble a female pattern of behavior. However, they displayed a greater sensitivity to the reinforcing properties of morphine than did females from the same experimental groups. In addition, although females also showed an altered response to morphine when compared to controls, they did not display an enhanced adrenal response to the CPP testing but exhibited permanently elevated basal levels of corticosterone. These data suggest that perinatal THC did not result either in feminization nor in masculinization of the behavioral and endocrine parameters studied. We might conclude from these findings that the proposed role of the HPA in the vulnerability to opiate reinforcement is sexually dimorphic, and that differential mechanisms may be underlying this response in both sexes.

The surprising efficacy of the low doses of THC for eliciting long-term behavioral alterations may be related to the well-documented biphasic actions of THC (Dewey, 1986) and anandamide (Fride et al., 1995), which commonly induce opposing actions at low doses (0.2 -2.0 mg/kg) when compared to higher ones (5-50 mg/kg). This has also been observed using anandamide, the proposed endogenous ligand for the brain cannabinoid receptor (Fride & Mechoulam, 1996a, 1996b; Wenger, Fragkakis, Giannikou, & Yiannakis, 1997). In any case, we observed that offspring of mothers exposed to THC 20 mg/kg, a dose that is 10-20 times higher than that estimated for human consumption of 2-3 marijuana cigarettes per day (Rosenkrantz et al., 1975), did not exhibit either the increased sensitivity to the reinforcing properties of morphine nor the altered response to novelty. However, they did display alterations in the locomotor activity (see Figures 2 and 3), in accordance with previous studies (Navarro et al., 1994a, 1996). Regardless of the lack of effects observed in the CPP paradigm, it is important to remark that other mechanisms, such as alterations in the ontogeny of cannabinoid receptors, nutritional deficits, or other adaptive processes may preclude the appearance of the effects observed with the lower doses.

In summary, perinatal exposure to THC doses related to human consumption produced a clear shift in the sensitivity to the reinforcing properties of morphine in male offspring. Interestingly, recent hypothesis attribute the onset of drug addiction to a drug-induced progressive dysregulation of homeostatic processes controlling behavioral, neuro-chemical, and endocrine responses to relevant external stimuli (Koob & Le Moal, 1997). The fact that cannabis consumption may result in dependence and addiction (Rodríguez de Fonseca et al., 1997) and that a recent report has linked a particular CB-1 receptor gene allele to intravenous use of opiates and psychostimulants (Comings et al., 1997) provide further support to the notion of cannabinoid exposure as a potential vulnerability factor for drug abuse.

References

Abel, E.L. (1980). Prenatal exposure to cannabis: a critical review of effects on growth, development, and behavior. *Behavioural and Neural Biology , 29*, 137-145.

Abood, M.E., & Martin, B.R. (1992). Neurobiology of marijuana abuse. *Trends in Neurosciences, 13*, 201-206.

Beltramo, M., Stella, N., Calignano, A., Lin, S.Y., Makriyannis, A., & Piomelli, D. (1997). Functional role of high-affinity

anandamide transport, as revealed by selective inhibition. *Science, 277*, 1094-1097.

Bonnin, A., de Miguel, R., Rodríguez-Manzaneque, J.C., Fernández-Ruiz, J.J., Santos, A., & Ramos, J.A. (1994). Changes in tyrosine hydroxylase gene expression in mesencephalic catecholaminergic neurons of immature and adult male rats perinatally exposed to cannabinoids. *Developmental Brain Research, 81*, 147-153.

Brake, S.C., Hutchings, D.E., Morgan, B., Lasalle, E., & Shi, T. (1987). Delta-9- tetrahydrocannabinol during pregnancy in the rat: II Effects on ontogeny of locomotor activity and nipple attachment in the offspring. *Neurotoxicology and Teratology, 9*, 45-49

Callaghan, P.M., Delagarza, R., Cunnighan, K.A., Henry, C., Kabbaj, M., Simon, H., Le Moal, M., & Maccari, S. (1994). Prenatal stress increases the hypothalamo-pituitary-adrenal axis response in young and adult rats. *Journal of Neuroendocrinology, 6*, 341- 345.

Comings, D.E., Muhleman, D., Gade, R., Johnson, P., Verde, R., Sucier, G., & MacMurray, J. (1997). Cannabinoid receptor gene (CNR1): association with i.v. drug use. *Molecular Psychiatry, 2*, 161-168.

Dalterio, S., & Bartke, A. (1979). Perinatal exposure to cannabinoids alters male reproductive function in mice. *Science, 205*, 1420-1422.

Day, N.L., Richardson, G.A., Goldschmidt, L., Robles, N., Taylor, P.M., Stoffer, D.S., & Cornelius, M.D. (1994). Effect of prenatal marijuana exposure on the cognitive development of offspring at age three. *Neurotoxicology and Teratology, 16*, 169-175.

Deminiere, J.M., Piazza, P.V., Guegan, G., Abrous, N., Maccari, S., Le Moal, M., & Simon, H. (1992). Increased locomotor response to novelty and propensity to intravenous amphetamine self-administration in adult offspring of stressed mothers. *Brain Research, 586*, 135-139.

Devane, W.A., Dysarz, F.A., Johnson, M.R., Melvin, L.S., & Howlett, A.C. (1988). Determination and characterization of a cannabinoid receptor in rat brain. *Molecular Pharmacology, 34*, 605-613.

Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., & Mechoulam, R. (1992).. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science, 258*, 1946-1949.

Dewey, W.L. (1986). Cannabinoid Pharmacology. Pharmacological Reviews 38, 151-178.

Di Marzo, V., Fontana, A., Cadas, H., Schinelli, S., Cimino, G., Schwartz, J.C., & Piomelli, D. (1994). Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature, 372*, 686-691.

Fernández-Ruiz, J.J., Muñoz, R.M., Romero, J., Villanúa, M.A., Makriyannis, A., & Ramos, J.A. (1997). Time-course of the effects of different cannabimimetics on prolactin and gonadotrophin secretion: evidence for the presence of CB-1 receptors in hypothalamic structures and their involvement in the effect of cannabimimetics. *Biochemical Pharmacology, 53*, 1919-1927.

Fernández-Ruiz, J.J., Rodríguez de Fonseca, F., Navarro, M., & Ramos, J.A. (1992). Maternal cannabinoid exposure and brain development: changes in the ontogeny of dopaminergic neurons In A. Bartke & L.L. Murphy (Eds.), *Neurobiology and neurophysiology of cannabinoids: Vol IV. Biochemistry and physiology of substance abuse* (pp. 119-164). Boca Raton, Florida, USA: CRC Press.

Fride, E., Barg, J., Levy, R., Saya, D., Heldman, I., Mechoulam, R., & Vogel, Z. (1995). Low doses of anandamides inhibit pharmacological effects of Δ^9-tetrahydro-cannabinol. *Journal of Pharmacology and Experimental Therapeutics, 272*, 699-707.

Fride, E., & Mechoulam, R. (1993). Pharmacological activity of the cannabinoid receptor agonist anandamide, a brain constituent. *European Journal of Pharmacology, 231*, 313-314.

Fride, E., & Mechoulam, R.(1996a). Developmental aspects of anandamide: ontogeny of response and prenatal exposure. *Psychoneuroendocrinology 21*, 157-172.

Fride, E., & Mechoulam, R.(1996b). Ontogenetic development of the response to anandamide and Δ^9-tetrahydrocannabinol in mice. *Developmental Brain Research , 95*, 131-134.

Fried, P.A. (1995). The Ottawa prenatal prospective study (OPPS): methodological issues and findings. It is easy to throw the baby out with the bath water. *Life Sciences, 56*, 2159- 2168.

Gagin, R., Kook, N., Cohen, E., & Shavit, Y. (1997). Prenatal morphine enhances morphine-conditioned place preference in adult rats. *Pharmacology Biochemistry and Behavior, 58*, 525-528.

Gaoni, Y., & Mechoulam, R. (1964). Isolation, structure elucidation, and partial synthesis of an active constituent of hashish. *Journal of American Chemical Society, 86*, 1646-1647.

García, L., de Miguel, R., Ramos, J.A., & Fernández-Ruiz, J.J. (1996). Perinatal-delta-9-tetrahydrocannabinol exposure in rats modifies the responsiveness of midbrain dopaminergic neurons in adulthood to a variety of challenges with dopaminergic drugs. *Drug and Alcohol Dependence, 42*, 155-166.

Gardner, E.L., & Lowinson, J.H. (1991). Marijuana's interaction with brain reward systems update 1991. *Pharmacology Biochemistry and Behavior, 40*, 571-580.

Gueudet, C., Santucci ,V., Rinaldi-Carmona, M., Soubrié, P., & Le Fur, G. (1995). The CB_1 cannabinoid receptor antagonist SR 141716A affects A_9 dopamine neuronal activity in the rat. *NeuroReport , 6*, 1293-1297.

Halikas, J.A., Weller, R.A., Mouse, C.L., & Hoffman, R.A. (1985). A longitudinal study of marijuana effects. *International Journal of Addiction, 20*, 701-711.

Hammer, R.P. Jr. (1985). The sex-hormone-dependent development of opiate receptors in the rat medial preoptic area. *Brain Research, 360*, 65-72.

Hand, T.H., Stinus, L., & Le Moal, M. (1989). Differential mechanisms in the acquisition and expression of heroin-induced place preference. *Psychopharmacology , 98*, 61-67.

Harfstrand, A., Fuxe, K., Cintra, A., Agnati, L.F., Zini, I., Wilkstrom, A.C., Okret, S., Zhao-Ying, Y., Goldstein, M., Steinbusch, H. Verhostad, A., & Gustaffson, J.A. (1986). Glucocorticoid receptor immunoreactivity in monoaminergic neurons in rat brain. *Proceedings National Academy of Sciences USA, 83*, 9779-9783.

Herkenham, M., Lynn, A.B., Little, M.D., Johnson, M.R., Melvin, L.S., De Costa, B.R., & Rice, K.C. (1990). Cannabinoid receptor localization in brain. *Proceedings National Academy of Sciences U.S.A., 87*, 1932-1936.

Holson, R.R., & Pearce, B. (1992). Principles and pitfalls in the analysis of prenatal treatment effects in multiparous species. *Neurotoxicology and Teratology, 14*, 221-228.

Hutchings, D.E., Martin, B.R., Gamagaris, Z., Miller, N., & Fico, T. (1989). Plasma concentrations of delta-9-tetrahydrocannabinol in dams and fetuses following acute or multiple prenatal dosing in rats. *Life Sciences, 44*, 697-701.

Hutchings, D.E., Morgan, B., Brake, S.C., Shi, T., & Lasalle, E. (1987). Delta-9-tetrahydrocannabinol during pregnancy in the rat: I. Differential effects on maternal nutrition, embryotoxicity, and growth in the offspring. *Neurotoxicology and Teratology, 9*, 39-43.

Insel, T.R., Kinsley, C.H., Mann, P.E., & Bridges, R.S. (1990). Prenatal stress has long- term effects on brain opiate receptors. *Brain Research , 511*, 93-97.

Jakubovic, A., Hattori, T., & Mc Geer, P.L. (1977). Radioactivity in suckled rats after giving ^{14}C-tetrahydrocannabinol to the mother. *European Journal of Pharmacology, 22*, 221-223.

Keller, R.W. Jr., Lefevre, R., Raucci, J., Carlson, J.N., & Glick, S.D. (1996). Enhanced cocaine self-administration in adult rats prenatally exposed to cocaine. *Neuroscience Letters, 205*, 153-156.

Keshet, G.I., & Weinstock, M. (1995). Maternal naltrexone prevents morphological and behavioral alterations induced in rats by prenatal stress. *Pharmacology Biochemistry and Behavior, 50*, 413-419.

Koob, G.F., & Le Moal, M. (1997). Drug abuse: hedonic homeostatic dysregulation. *Science, 278*, 52-58.

Kumar, A.M., Haney, M., Becker, T., Thompson, M.L., Kream, R.M., & Miczek, K. (1990). Effect of early exposure to delta-9-tetrahydrocannabinol on the levels of opioid peptides, gonadotrophin-releasing hormone, and substance P in the adult male rat brain. *Brain Research*, 525, 78-83.

Lee, S., & Rivier, C. (1996). Gender differences in the effect of prenatal alcohol exposure on the hypothalamic-pituitary-adrenal axis response to immune signals. *Psychoneuroendocrinology, 21*, 145-155.

Maccari, S., Piazza, P.V., Deminiere, J.M., Lemaire, V., Mormede, P., Simon, H., Angelucci, L., & Le Moal M (1991). Life events-induced decrease of corticosteroid type-I receptors is associated with reduced corticosterone feedback and enhanced vulnerability to amphetamine self-administration. *Brain Research, 547*, 7-12.

Martín, S., Crespo, J.A., Ferrado, R., García-Lecumberri, C., Gil, L., Ramos, J.A., Fernández-Ruiz, J.J., Diez, N., Manzanares, J., & Ambrosio, E. (1996). Effects of Delta-9-THC perinatal treatment of mothers on morphine and food operant reinforced behaviors in the adult offspring. *Society for Neuroscience Abstract, 22*, 167.

Martín-Calderón, J.L., Muñoz, R.M., Villanúa, M.A., del Arco, I., Moreno, J.L., Rodríguez de Fonseca, F., & Navarro, M. (1998). Characterization of the acute endocrine actions of HU-210, a potent synthetic cannabinoid in rats. *European Journal of Pharmacology* (in press).

Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.L., & Bonner, T.I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature, 346*, 561-564.

McCormick, C.M., Smythe, J.W., Sharma, S., & Meaney, M. (1995). Sex-specific effects of prenatal stress on hypothalamic-pituitary-adrenal responses to stress and brain glucocorticoid receptor density in adult rats. *Brain Research, 84*, 55-61.

McEwen, B.S. (1987). Steroid hormones and brain development: some guidelines for understanding actions of pseudohormones and other toxic agents. *Environmental and Health Perspectives, 74*, 177-192.

Mechoulam, R. (1986). The pharmacohistory of Cannabis sativa. In R. Mechoulam (Ed.), *Cannabinoids as Therapeutic Agents* (pp. 1-19). Boca-Raton, Florida, USA. CRC Press.

Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N.E., Schatz, A.R., Gopher, A., Almog, S., Martin, B.R., Compton, D.R., Pertwee, R.G., Griffin, G., Bayewitch, M., Barg, J., & Vogel, Z. (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochemical Pharmacology, 50*, 83-90.

Mechoulam, R., Lander, N., Srebnik, M., Breuer, A., Segal, M., Feigenbaum, J.J., Jarbe, T.U.C., & Consroe, P. (1987). Stereochemical requirements for cannabimimetic activity. In R.S. Rapaka & A. Makriyannis (Eds.), *Structure-activity relationships of the cannabinoids* (pp. 15-30). Rockville, MD, USA. NIDA research monograph 79.

Mirmiran, M., & Swaab, D.F. (1987). Influence of drugs on brain neurotransmission and behavioral stages during development. *Development of Pharmacology Therapeutics, 10*, 377-384.

Molina, V.A., Wagner, J.M., & Spear, L.P. (1994). The behavioral response to stress is altered in adult rats exposed perinatally to cocaine. *Physiology and Behavior, 55*, 941-945.

Molina-Holgado, F., Molina-Holgado, E., Leret, M.L., González, M.I., & Reader, T.A. (1993). Distribution of indoleamines and [3H]-paroxetine binding in rat brain region following acute or perinatal delta-9-tetrahydrocannabinol treatments. *Neurochemical Research, 18*, 1183-1191.

Navarro, M., Fernández-Ruiz, J.J., de Miguel, R., Hernández, M.L., Cebeira, M., & Ramos, J.A. (1993a). An acute dose of Δ^9-tetrahydrocannabinol affects behavioral and neurochemical indices of mesolimbic dopaminergic activity. *Behavioural Brain Research, 5*, 37-46.

Navarro, M., Fernández-Ruiz, J.J., de Miguel, R., Hernández, M.L., Cebeira, M., & Ramos, J.A. (1993b). in Motor disturbances induced by an acute dose of Δ^9-tetrahydro-cannabinol: possible involvement of nigrostriatal dopaminergic alterations. *Pharmacology Biochemistry and Behavior, 45*, 291-298.

Navarro, M., Hernández, E., Muñoz, R.M., del Arco, I., Villanúa, M.A., Carrera, M.R.A., & Rodríguez de Fonseca, F. (1997). Acute administration of the CB-1 cannabinoid receptor

antagonist SR141716A induces anxiety-like responses in the rat. *NeuroReport, 8*, 491-496.

Navarro, M., Martín-Calderón, J.L., del Arco, I., Villanúa, M.A., Sánchez, L., Muñoz, R.M., Orengo, F., Capdevila, E., Chowen, J., & Rodríguez de Fonseca, F. (in press). Effects of subchronic treatment with dopaminergic agonists and antagonists on the acute sensitivity to cannabinoid exposure: behavioral, neurochemical and hormonal studies. In T. Archer, R. Beninger, & T. Palomo (Eds.), *Strategies for Studying Brain Disorders*, Vol. IV. Madrid: Cerebro y Mente.

Navarro, M., de Miguel, R., Rodríguez de Fonseca, F., Ramos, J.A., & Fernández-Ruiz, J.J. (1996). Perinatal cannabinoid exposure modifies the sociosexual approach behavior and the mesolimbic dopaminergic activity of adult male rats. *Behavioural Brain Research, 75*, 91-98.

Navarro, M., Rodríguez de Fonseca, F., Hernández, M.L., Ramos, J.A., & Fernández-Ruiz, J.J. (1994a). Changes in the adult motor behavior following perinatal cannabinoid exposure in rats: involvement of nigrostriatal dopaminergic activity. *Pharmacology Biochemistry and Behavior, 47*, 47-58.

Navarro, M., Rubio, P., & Rodríguez de Fonseca, F. (1994b). Sex-dimorphic psychomotor activation after perinatal exposure to (-)-Δ^9-tetrahydrocannabinol. An ontogenic study in wistar rats. *Psychopharmacology, 116*, 414-422.

Navarro, M., Rubio, P., & Rodríguez de Fonseca, F. (1995). Behavioural consequences of maternal exposure to cannabinoids in rats. *Psychopharmacology, 122*, 1-14.

Piazza, P.V., Deminiere, J.M., Le Moal, M., & Simon, H. (1989). Factors that predict individual vulnerability to amphetamine self-administration. *Science, 245*, 1511-1513.

Piazza, P.V., Deroche, V., Deminiére, J.M., Maccari, S., Le Moal, M., & Simon, H. (1993). Corticosterone in the range of stress-induced levels possesses reinforcing properties: Implications for sensation-seeking behaviors. *Proceedings National Academy of Sciences USA, 90*, 11738-11742.

Piazza, P.V., & Le Moal, M. (1996). Pathophysiological basis of vulnerability to drug abuse: role of an interaction between stress, glucocorticoids, and dopaminergic neurons. *Annual Review of Pharmacology and Toxicology, 36*, 359-378

Poltyrev, T., & Weinstock, M. (1997). Effect of prenatal stress on opioid component of exploration in different experimental situations. *Pharmacology Biochemistry and Behavior, 58*, 387-393.

Rinaldi-Carmona, M., Barth, F., Heaulme, M., Shire, D., Calandra, B., Congy, C., Martinez, S., Maruani, J., Neliat, G., Caput, D., Ferrara, P., Soubrie, P., Breliere, J.C., & Le Fur, G. (1994). SR 141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS letters, 350*, 240-244.

Robins, L.N., & Mills, J.L. (1993). Effects of in utero exposure to street drugs. *American Journal of Public Health*, supplement to Vol 83, 8-32.

Rodríguez de Fonseca, F., Carrera, M.R.A., Navarro, M., Koob, G.F., & Weiss, F. (1997). Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. *Science, 276*, 2050-2054.

Rodríguez de Fonseca, F., Cebeira, M., Fernández-Ruiz, J.J., Navarro, M.., & Ramos, J.A. (1991a). Effects of pre- and perinatal exposure to hashish extracts on the ontogeny of brain dopaminergic neurons. *Neuroscience, 43*, 713-723.

Rodríguez de Fonseca, F., Cebeira, M., Hernández, M.L., Ramos, J.A., & Fernández-Ruiz, J.J. (1990). Changes in brain dopaminergic indices induced by perinatal exposure to cannabinoids in rats. *Developmental Brain Research, 51*, 237-24

Rodríguez de Fonseca, F.A., Cebeira, M., Ramos, J.A., Martín, M., & Fernández-Ruiz, J.J. (1994b). Cannabinoid receptors in rat brain areas: sexual differences, fluctuations during estrous cycle, and changes after gonadectomy and sex steroid replacement. *Life Sciences, 54*, 159-170.

Rodríguez de Fonseca, F., Fernández-Ruiz, J.J., Eldridge, J.C., Steger, R.W., Bartke, A., & Murphy, L.L. (1991b). Effects of the exposure to delta-9-tetrahydro-cannabinol on the adrenal medullary function: evidence of an acute effect and development of tolerance in chronic treatments. *Pharmacology Biochemistry and Behavior, 40*, 593-598.

Rodríguez de Fonseca, F., Fernández-Ruiz, J.J., Murphy, L.L., Cebeira, M., Steger, R.W., Bartke, A., & Ramos, J.A. (1992b). Acute effects of Δ^9-tetrahydrocannabinol on dopaminergic activity in several rat brain areas. *Pharmacology Biochemistry and Behavior, 42*, 269-275.

Rodríguez de Fonseca, F., Hernández, M.L., de Miguel, R., Fernández-Ruiz, J.J., & Ramos, J.A. (1992a). Early changes in the development of dopaminergic neurotransmission after maternal exposure to cannabinoids. *Pharmacology Biochemistry and Behavior, 41*, 469-474.

Rodríguez de Fonseca, F., Martín-Calderón, J.L., Mechoulam, R., & Navarro, M. (1994a). Repeated stimulation of D1 dopamine receptors enhances (-)-11-hydroxy-Δ^8-tetrahydrocannabinol-dimethylheptyl-induced catalepsy in male rats. *NeuroReport, 5*, 761-765.

Rodríguez de Fonseca, F., Ramos, J.A., Bonnin, A., & Fernández-Ruiz, J.J. (1993). Presence of cannabinoid binding sites in the brain from early postnatal ages. *NeuroReport, 4*, 135-138.

Rodríguez de Fonseca, F., Rubio, P., Martín-Calderón, J.L., Caine, S.B., Koob, G.F., & Navarro, M. (1995a). The dopamine receptor agonist 7-OH-DPAT modulates the acquisition and expression of morphine-induced place preference. *European Journal of Pharmacology, 274*, 47-55.

Rodríguez de Fonseca, F., Rubio, P., Menzaghi, F., Merlo-Pich, E., Rivier, J., Koob, G.F., & Navarro, M. (1996). Corticotropin-releasing factor (CRF) antagonist [D- Phe12, Nle21,38, CMeLeu37] CRF attenuates the acute actions of the highly potent cannabinoid receptor agonist HU-210 on defensive-withdrawal behavior in rats. *Journal of Pharmacology and Experimental Therapeutics, 276*, 56-64.

Rodríguez de Fonseca, F., Villanúa, M.A., Muñoz, R.M., San-Martin-Clarke, O., & Navarro, M. (1995b). Differential effects of chronic treatment with either dopamine D1 or D2 receptor agonists on the acute neuroendocrine actions of the highly potent synthetic cannabinoid HU-210 in male rats. *Neuroendocrinology, 61*, 714-721

Romero, J., García-Palomero, E., Lin, S., Ramos, J.A., Makriynnis, A., & Fernández-Ruiz, J.J. (1996). Extrapyramidal effects of methanandamide, an analogue of anandamide, the endogenous CB1 receptor ligand. *Life Sciences, 58*, 1249-1257.

Rosenkrantz, H., Sprague, R.A., Fleischman, R.W., & Braude, M.C. (1975). Oral Δ^9-tetrahydrocannabinol toxicity in rats treated for periods up to six months. *Toxicology and Applied Pharmacology, 32*, 399-417.

Rubio, P., Rodríguez de Fonseca, F., Martín- Calderón, J.L. del Arco, I., Bartolomé, S., Villanúa, M.A., & Navarro, M. (In press). Maternal exposure to low doses of Δ^9-tetrahydrocannabinol facilitates morphine-induced place conditioning in adult male offspring. *Pharmacology Biochemistry and Behavior.*

Rubio, P., Rodríguez de Fonseca, F., Muñoz, R.M., Ariznavarreta, C., Martín- Calderón, J.L., & Navarro, M. (1995). Long-term behavioral effects of perinatal exposure to Δ^9-tetrahydrocannabinol in rats: possible role of pituitary-adrenal axis. *Life Sciences, 56*, 2169-2176.

Shaham, Y., & Stewart, J. (1995). Stress reinstates heroin-seeking in drug-free animals: an effect mimicking heroin, not withdrawal. *Psychopharmacology, 119*, 334-341.

Stella, N., Schweitzer, P., & Piomelli, D. (1997). A second endogenous cannabinoid that modulates long-term potentiation. *Nature, 388*, 773-778.

Taylor, A.N., Branch, B.J., Nelson, L.R., Fane, L.A., & Poland, R.E. (1986). Prenatal ethanol and ontogeny of pituitary-adrenal responses to ethanol and morphine. *Alcohol, 3*, 255-259.

Valleé, M., Mayo, W., Dellu, F., Le Moal, M., Simon, H., & Maccari (1997). Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: correlation with stress-induced corticosterone secretion. *The Journal of Neuroscience, 17*, 2626-2636.

Vela, G., Fuentes, J.A., Bonnin, A., Fernandez-Ruiz, J., & Ruiz-Gayo, M. (1995). Perinatal exposure to Δ^9-tetrahydrocannabinol leads to changes in opioid-related behavioral patterns in rats. *Brain Research, 680*, 142-147.

Walters, D.E., & Carr, L.A. (1986). Changes in brain catecholamine mechanisms following perinatal exposure to marihuana. *Pharmacology Biochemistry and Behavior, 25*, 763-778.

Ward, I.L., & Weisz. J. (1984). Differential effects of maternal stress on circulating levels of corticosterone, progesterone and testosterone in male and female rat fetuses and their mothers. *Endocrinology, 114*, 1635-1644.

Wenger, T., Fragkakis, G., Giannikou, P., & Yiannakis, N. (1997). The effects of prenatally administered endogenous cannabinoid on rat offspring. *Pharmacology Biochemistry and Behavior, 58*, 537-544.

Zuckerman, B. (1991). Drug effects - A search for mechanisms. In M.N. Kilbey and K. Asghar (Eds.), Methodological issues in controlled studies on effects of prenatal exposure to drug abuse (pp 352-362). *NIDA Research Monograph* 114.

Received October 6, 1997
Revision received February 12, 1998
Accepted March 9, 1998