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Effects of prenatal exposure to delta-9-tetrahydrocannabinol on reproductive, endocrine and immune parameters of male and female rat offspring

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The effects of prenatal THC administration, given during the third week of gestation in rats, on the reproductive, endocrine and immune systems of the adult offspring were examined. THC treatment blocked the surge of testosterone which occurs in the male rat fetus on gestation day 18. Moreover, when copulatory parameters were measured in adult male offspring, males that had been exposed to THC *in utero* exhibited an increased latency to mount (THC: 245 ± 49 *vs* vehicle: 99 ± 12 sec) and none of the males ejaculated. Female rats exposed to THC *in utero*, exhibited an increased incidence of irregular estrous cycles and the number of females exhibiting lordosis behavior was reduced when compared to vehicle controls. Hormone analyses revealed that prolactin levels were significantly lower in the THC- *vs* vehicle-exposed male (THC: 5.2 ± 0.4 *vs* vehicle: 8.4 ± 0.6 ng/ml) and female offspring (THC: 5.7 ± 0.3 *vs* vehicle: 12.2 ± 1.8 ng/ml). However, there were no significant differences in basal plasma LH levels or in testicular weights of the male offspring. Thymus weight and total number of thymocytes were significantly higher in THC-exposed male and female rats when compared to vehicle controls. Together, these results indicate that maternal THC exposure has long-lasting effects on reproductive, endocrine and immune parameters of both male and female rat offspring.

Keywords: Delta-9-tetrahydrocannabinol; pregnancy; copulatory behavior; immune; testosterone; prolactin

Introduction

Considerable research efforts have demonstrated that marijuana, and in particular the major psychoactive constituent of marijuana, delta-9-tetrahydrocannabinol (THC), have profound effects on the reproductive and immune systems of humans and laboratory animals. Smoking marijuana or injections of THC is associated with decreased luteinizing hormone (LH; Marks, 1973; Besch *et al.*, 1977; Tyrey, 1978; Murphy *et al.*, 1990) and prolactin secretion (Kramer & Ben-David, 1978; Asch *et al.*, 1979; Hughes *et al.*, 1981), decreased testosterone in males (Kolodny *et al.*, 1974; Smith *et al.*, 1976; Steger *et al.*, 1990), and alterations in gonadotropin and ovarian steroid secretion in females with accompanying changes in cyclicity (Smith *et al.*, 1983; Mendelson *et al.*, 1985, 1986). Marijuana and THC have also been shown to be immunosuppressive, altering both the humoral- and cell-mediated responses of humans or animals (Petersen *et al.*, 1976; Zimmerman *et al.*, 1977). Indeed, immune alterations associated with marijuana or THC include a reduced proliferative response to mitogens (Daul & Heath, 1975; Petersen *et al.*, 1976; Pross *et al.*, 1987), inhibition of phagocytic function (Petersen *et al.*, 1974), interferon production (Blanchard *et al.*, 1986), natural killer cell activity (Daul & Health, 1975), and function of cytotoxic T cells (Klein *et al.*, 1991), exacerbated viral infections (Cabral *et al.*, 1986) and reduced resistance to bacterial pathogens (Morahan *et al.*, 1979; Blanchard *et al.*, 1986).

Although most studies have been concerned with the effects of cannabinoids on reproductive and immune parameters in adult animals, relatively little is known about the effects of THC administration to pregnant animals on the reproductive and immune systems of the adult male and female offspring. Perinatal THC exposure has been shown to decrease (Dalterio *et al.*, 1984), increase (Dalterio, 1980) or have no effect on plasma LH levels (Dalterio *et al.*, 1984a) in intact male mice depending on the timing of cannabinoid exposure during the perinatal period. When THC was administered to pregnant mice on gestation days (GD) 12-16, testosterone levels were significantly reduced in the adult male offspring (Dalterio & Bartke, 1981). Moreover, perinatal THC exposure has also been shown to significantly inhibit copulatory behavior in male mice (Dalterio, 1980). Newborn female rats injected with THC during the first 5 days after birth, exhibited constant diestrous vaginal smears or irregular estrous cycle (Kumar *et al.*, 1986). In addition, serum LH levels were significantly reduced when compared to control females. The effect of prenatal THC exposure on the immune system of the offspring is virtually unknown. One early study has shown that maternal exposure of mice to cannabinol, a relatively nonpsychoactive component of marijuana, on day 1 post-partum produced an increase in γ-globulin concentrations in the adult male offspring (Dalterio *et al.*, 1986).

In the present study, THC was administered to pregnant rats during the last week of gestation, a critical period during which sexual differentiation of the neuroendocrine axes (McLusky & Naftolin, 1981) and significant developmental changes in the immune system have been shown to occur (Kalland *et al.*, 1978). This study was designed to provide new information on whether exposure to THC during late fetal development can lead to long-lasting effects on endocrine, reproductive and immune parameters of the adult male and female offspring.

Results

Plasma levels of THC were undetectable in vehicle-treated animals, however relatively high levels of THC were measured in both dam and fetal blood samples obtained on GD 17, 18 and 19 in THC-treated animals (Figure 1). Interestingly, on postnatal day 1 (PND1) or the day of birth, approximately 3 days following the last THC dose, THC levels were detected in maternal and fetal blood samples. There were no changes in body weight of the fetuses, one day old pups or dams (Table 1) or in the length of gestation period (22 days) and litter size (veh: 13 ± 0.6 *vs* THC: 13.7 ± 1 pups/litter) as a result of THC exposure when compared to vehicle-treated controls.

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THC significantly reduced testosterone levels on GD 17, 18 and 19 in male fetuses when compared to vehicle controls and effectively blocked the surge of testosterone which occurs in the male fetus on GD 18 and 19 (Figure 2). In the dam, there was a small rise in plasma testosterone concentrations on GD 18 and 19 in vehicle controls; however, in THC-treated dams, testosterone levels were significantly reduced ($P<0.05$) on GD 18 when compared to vehicle-treated animals (Figure 2).

When copulatory behavior was examined in the male offspring of vehicle- and THC-treated pregnant rats, the latency to mount was significantly increased in animals exposed prenatally to THC when compared to vehicle controls (Table 2). There was also an apparent increase in intromission latency, but it was not statistically significant. None of the THC-exposed animals ejaculated during the 30 min test, suggesting that THC exposure *in utero* had altered normal copulatory behavior in the male. Hormone analyses revealed that plasma prolactin levels were significantly lower ($P<0.05$) in THC- *vs* vehicle-exposed male and female rats (Figure 3). However, there were no significant differences in basal plasma LH levels (THC: 0.33 ± 0.04 *vs* vehicle: 0.37 ± 0.04 ng/ml) or in total testicular weights (THC: 930 ± 30 *vs* vehicle 918 ± 16 mg/100 g b.w.) in the male offspring.

Starting at 45 days of age, the adult female offspring were examined daily to determine if they had normal estrous cycles. In females exposed to THC *in utero*, the number of animals exhibiting a 4-day cycle was decreased (vehicle: 7/12; THC: 0/12), however there was a slight but insignificant increase in the number of 5-day cycles (vehicle: 4/12; THC: 6/12) and a greater number of THC-exposed females that exhibited irregular estrous cycles (vehicle: 1/12; THC: 6/12). Moreover, the number of THC-exposed rats exhibiting lordosis behavior was significantly reduced when compared to vehicle controls (vehicle: 90% *vs* THC: 25%; $P<0.05$). The latency to first lordosis in those THC-exposed animals which did exhibit lordosis behavior was significantly increased compared to vehicle controls (vehicle: 138 ± 60 *vs* THC: 508 ± 48 sec; $P<0.05$). The LQ appeared reduced in THC-exposed animals but this difference was not statistically significant (vehicle: 43 ± 10% *vs* THC: 20 ± 10%).

As the effects of prenatal THC exposure on the immune system of the offspring had not been studied, thymus and spleen weights and cellularity in vehicle- and THC-exposed intact male and female rats were measured. Interestingly, thymus weight and total number of thymocytes were significantly higher in THC-exposed male and female rats (Table 3) when compared to vehicle controls. Spleen weights and number of splenocytes were also slightly higher in the male and female animals exposed to THC, but statistical significance was not achieved.

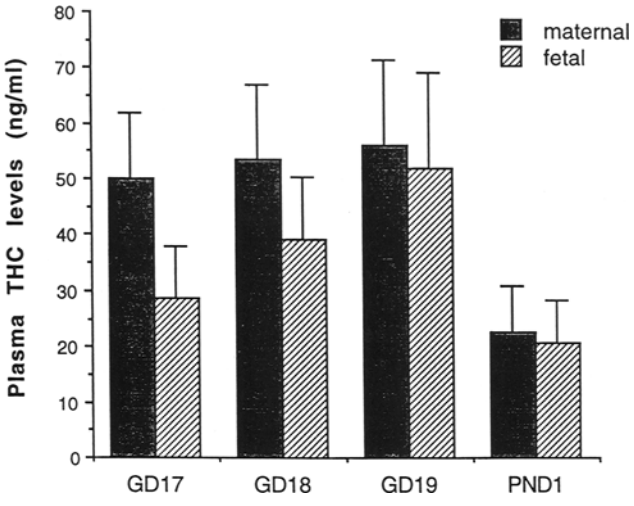

Figure 1 THC levels in maternal (solid bars) and fetal (striped bars) plasma samples obtained on GD 17, 18 or 19 and on PND 1

Table 1 Body weights during and after treatment with vehicle or THC

		Vehicle	*THC*
dam	(GD 14)	281 ± 14	278 ± 7
	(GD 16)	292 ± 14	288 ± 7
	(GD 18)	310 ± 16	309 ± 9
	(GD 20)	353 ± 16	349 ± 9
fetal	(GD 17)	.457 ± .01	.454 ± .01
	(GD 18)	.784 ± .02	.786 ± .02
	(GD 19)	1.39 ± .02	1.38 ± .02
pup	(PND 1)	6.69 ± .05	6.85 ± .06

Discussion

This is the first study to show that the administration of THC to pregnant rats during the last week of pregnancy, effectively blocks the GD 18 surge of testosterone in male fetuses and inhibits copulatory behavior and prolactin secretion in the adult offspring. Early work with rodents has shown that the perinatal hormonal milieu is critical for

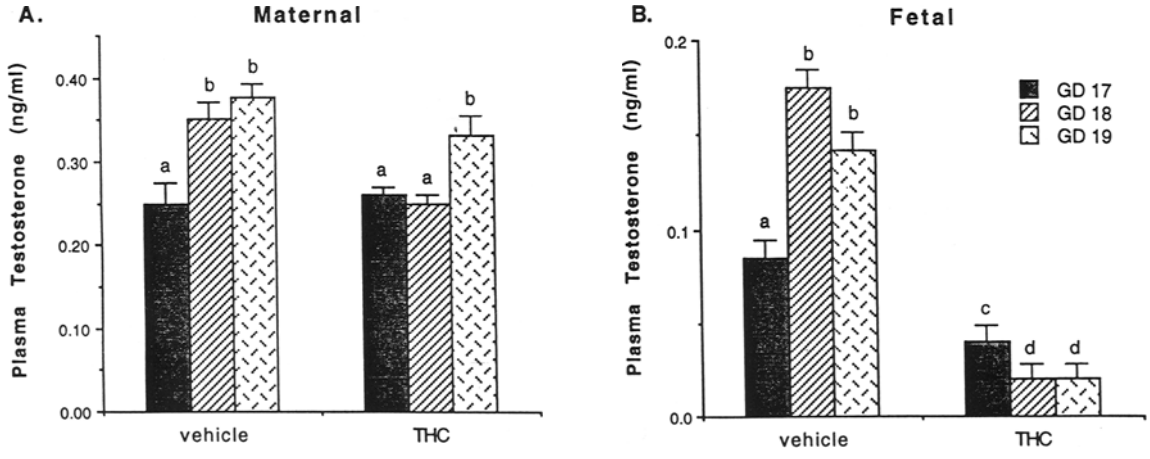

Figure 2 Testosterone levels from dams (graph A) and GD 17, 18 and 19 day old male fetuses (graph B) exposed to oil vehicle (control) or THC on GD 14–19. Bars represent mean ± SEM. Differing letters above bars denote significance at $p<0.05$. N = 6 animals/group

sexual differentiation of the brain as expressed by the pattern of gonadotropin secretion and sexual behavior (Gorski, 1971; McLusky & Naftolin, 1981). It is thought that the fetal testosterone surge that occurs on GD 18 in male rats, acts on the brain to organize the development of sexually dimorphic neural structures that underlie sex differences in behavior and gonadotropin secretion (Wilson *et al.*, 1983). Therefore, the ability of THC to suppress male fetal testosterone levels may lead to the decrease in copulatory behavior exhibited by these animals as adults.

The suppression in fetal testosterone levels by THC may involve inhibition of fetal LHRH and LH secretion (Murphy *et al.*, 1990a; Wenger *et al.*, 1992), inhibition of testicular androgen production (Jakubovic *et al.*, 1979) and/or stimulation of a component of the hypothalamic-pituitary-adrenal axis which can lead to suppression of LH release (Dewey *et al.*, 1970; Rivier & Rivest, 1991). THC is a potent stimulator of adrenocorticotropin and corticosterone release (Dewey *et al.*, 1970; Rodríguez de Fonseca *et al.*, 1992; Weidenfeld *et al.*, 1994) and may also have estrogen-like effects (Solomon *et al.*, 1975). Perinatal exposure to glucocorticoids or estrogens during the critical period of CNS sexual development has been associated with altered gonadotropin secretion and sexual behavior parameters in the adult male and female offspring (Reinisch, 1974; Herrenkohl, 1979). In the present study, female offspring exposed to THC *in utero* exhibited an increased incidence of irregular estrous cycles and the number of females exhibiting lordosis behavior was reduced when compared to vehicle controls. The cyclicity and altered ability to respond to steroid priming and exhibit lordosis behavior in prenatally THC-exposed females may be a consequence of the ability of THC to enhance glucocorticoid secretion and/or elicit estrogen-like responses in the fetus, thus permanently affecting brain sites regulating reproduction in the female.

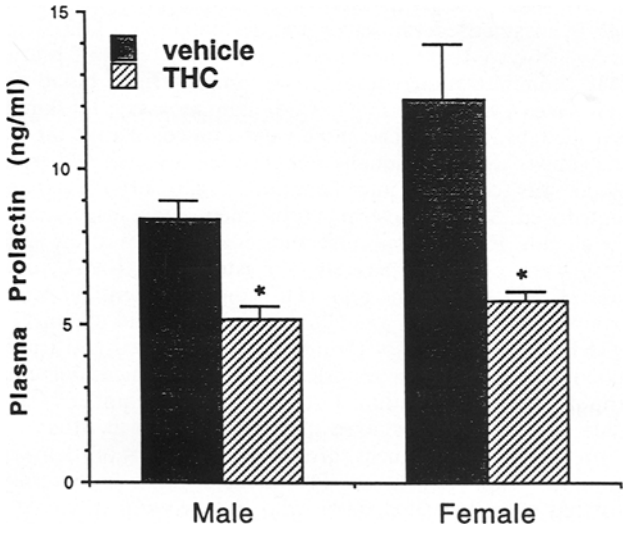

Figure 3 Plasma prolactin levels from adult male and female offspring exposed to either vehicle of THC *in utero*. * = $p < 0.05$

THC has been shown to readily cross the placenta (Harbison & Mantilla-Plata, 1972) and in the present study comparable THC levels were measured in maternal and fetal blood samples. Interestingly, measurable levels of THC were found in maternal and fetal blood on PND 1, approximately 72 h following the last administered dose of THC, suggesting that residual THC during the postnatal period may also be contributing to the long-lasting effects of THC in the adult. Indeed, perinatal THC exposure has been shown to cause irregular estrous cyclicity and altered copulatory behavior, gonadotropin secretion and neurotransmitter function in adult rodent offspring (Dalterio *et al.*, 1984a; Kumar *et al.*, 1986; Walters & Carr, 1986; Rodríguez de Fonseca *et al.*, 1991). That the developing dopaminergic system is particularly vulnerable to perinatal cannabinoid exposure (Rodríguez de Fonseca *et al.*, 1991), may explain in part our findings that prolactin levels were reduced in THC-exposed male and female offspring. Indeed, these earlier studies have shown an increase in hypothalamic tyrosine hydroxylase activity and dopamine concentrations and a decrease in prolactin levels in rats exposed to cannabinoids during the perinatal period (Rodríguez de Fonseca, 1991).

An important finding in this study was that the immune system may be affected as a result of fetal exposure to THC. A significant increase in thymus weight and number of thymocytes was measured in both male and female adult offspring exposed to THC during the third week of gestation. It is not known whether the change in lymphoid tissue weight in this study reflects potential alterations in immune function.

Table 2 Copulatory behavior parameters in male rats exposed to THC or vehicle *in utero*

	ML (sec)	*IL (sec)*	*EL (sec)*	*PEI (sec)*	*%E*
vehicle (n = 12)	99 ± 12	342 ± 102	1250 ± 215	248 ± 76	50
THC (n = 12)	245 ± 49*	615 ± 128	–	–	0

Mount latency (ML), Intromission latency (IL), Ejaculation latency (EL), Post-ejaculatory Interval (PEI), Percent animals ejaculating (%E); * = $p < 0.05$ *versus* vehicle control.

Table 3 Effect of *in utero* exposure to THC or its vehicle on thymus, spleen and body weight and number of whole tissue thymocytes and splenocytes from 75 day old male offspring

Rx (*n* = 6)	*body wt. (g)*	*thymus wt. (mg/100 g b.w.)*	*spleen wt. (mg/100 g b.w.)*	*thymocytes* ($\times 10^8$/100 g b.w.)	*splenocytes* ($\times 10^7$/100 g b.w.)
Vehicle	377 ± 7	160.3 ± 2.2	195.4 ± 5.5	4.9 ± .2	5.4 ± 0.4
THC	375 ± 8	184.5 ± 4.0*	226.1 ± 5.9	6.2 ± .3*	6.2 ± 0.4

mean ± SEM; * = $p < 0.05$.

Table 4 Effect of *in utero* exposure to THC or its vehicle on thymus, spleen and body weight and number of whole tissue thymocytes and splenocytes from 75 day old female offspring

Rx (*n* = 6)	*body wt. (g)*	*thymus wt. (mg/100 g b.w.)*	*spleen wt. (mg/100 g b.w.)*	*thymocytes* ($\times 10^8$/100 g b.w.)	*splenocytes* ($\times 10^7$/100 g b.w.)
Vehicle	234 ± 5	154.4 ± 4.0	236.0 ± 6.9	4.6 ± .1	9.7 ± 1.8
THC	224 ± 7	180.7 ± 6.9*	252.5 ± 8.6	6.4 ± .3*	13.7 ± 2.4

mean ± SEM; * = $p < 0.05$.

However, neonatal treatment with glucocorticoids or estrogens, which suppress immune function during development and in the adult, have been shown to decrease lymphoid tissue weight in the immature rodent followed by an increase in tissue weight, perhaps reflecting a rebound effect in the adult animal (Luster *et al.*, 1979; Blair, 1981; Laceta *et al.*, 1988). In only one other study have the effects of perinatal cannabinoids on the immune system of the adult been examined. In mice, maternal exposure to cannabinol on day 1 post-partum resulted in increased levels of γ-globulin concentrations in the adult male offspring suggesting an immunostimulatory effect in perinatally cannabinol-exposed mice (Dalterio *et al.*, 1986).

Whether the THC-induced alterations in reproductive, endocrine and immune parameters in the current study are an indirect result of THC-induced changes in the hormonal milieu during the perinatal period and/or a direct effect of THC on the immune system is not known. A complex interrelationship exists between the endocrine and immune axes (Healy *et al.*, 1983; Grossman, 1984; Bateman *et al.*, 1989) and drugs capable of disrupting the hormonal milieu during the perinatal period may interfere with normal development of both the reproductive and immune systems. Indeed, manipulation of sex steroid concentrations during pre- or perinatal development has been shown to alter copulatory behavior parameters (Gorski, 1971; McLusky & Naftolin, 1981), gonadotropin secretion (Gorski, 1971) and immune function (Grossman, 1984) in laboratory animals. In the current study, prenatal THC administration, which altered maternal and fetal testosterone levels, caused both male and female adult offspring to exhibit decreased copulatory behavior, lowered prolactin levels and increased thymus weight. Furthermore, the immune system itself has been shown to play a role in normal reproductive development. Studies with neonatally thymectomized rats or congenitally athymic nude mice have demonstrated that these animals exhibit a significant delay in the onset of puberty (Besedovsky & Sorkin, 1974) and impaired gonadotropin secretion (Michael *et al.*, 1980). Moreover, the critical period for thymectomy to affect reproduction in rodents (Nishizuka & Sakakura, 1971) parallels the timing of hormone-dependent sexual differentiation of the brain that results in sex-specific patterns of gonadotropin release and behavior (Gorski, 1971; McLusky & Naftolin, 1981). Although it is not known whether THC is able to directly affect thymic function in the developing animal, studies have indicated the presence of cannabinoid receptors in components of the immune system of adult animals (Kaminski *et al.*, 1992) suggesting that cannabinoids can directly modulate the immune system.

In conclusion, these data indicate that the administration of THC to pregnant rats during the last week of gestation can have long-lasting effects on the reproductive, endocrine and immune systems of both male and female offspring. Whether alterations in the maternal and fetal hormone milieu or cannabinoid effects on lymphoid tissue function are responsible for the ability of prenatal THC exposure to elicit these effects in adult animals will be investigated in future studies.

Materials and methods

Animals Adult female Sprague-Dawley rats of the Hsd(SD)BR strain were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis) at a body weight of 150–175 gm and individually housed in the Southern Illinois University-Carbondale vivarium in environmentally controlled animal quarters illuminated by fluorescent lighting 12 h each day (06.00–18.00 h). Teklad laboratory chow and water were provided *ad libitum*. Females were individually placed with proven breeder male Sprague-Dawley rats also obtained from Harlan Sprague-Dawley, Inc, and daily vaginal smears were examined for the detection of sperm; gestation day 1 (GD 1) was defined as the first morning a sperm-positive smear was detected. Experimental protocols were performed in accordance with the regulations in the 'Guide for the Care and Use of Laboratory Animals' published by the Public Health Service.

Treatment Immediately before use, delta-9-tetrahydrocannabinol (THC), obtained in an ethanol solution from the National Institute on Drug Abuse, was added to sesame oil vehicle (Sigma, St. Louis) and the ethanol was evaporated at 45–50°C. Experimental female rats received two daily doses of 5.0 mg/kg b.w. THC *per os* in a volume of 0.5 ml/kg b.w. Control female rats were treated with sesame oil vehicle. For oral administration, a blunted 3/4 inch, 23 gauge needle connected to a glass syringe was gently inserted in the animal's mouth and the THC or sesame oil vehicle was administered (Murphy *et al.*, 1990a; Gher, 1992). THC or vehicle was administered twice daily at 09.00 h and at 17.00 h beginning on GD 14 and ending on the evening of GD 19.

Experiments Pregnant rats (n = 4/treatment group) were lightly anesthetized with ether and decapitated on GD 17, 18 or 19 between 10.00 and 11.00 h (approximately 1–2 h after THC administration) in order to obtain trunk blood for THC measurements using a radioimmunoassay kit kindly provided by NIDA. The uteri were excised, placed on ice, and fetuses were individually removed, decapitated and trunk blood was collected into heparinized capillary tubes and centrifuged. Sex was determined by microscopic inspection of the gonads. Plasma from male and female fetuses of the same litter were pooled separately and stored at −40°C until assayed for testosterone and THC concentrations. A second group of treated dams was allowed to deliver and within 12 h of delivery, dams and offspring were anesthetized and trunk-blood was obtained for testosterone and THC measurements. The duration of gestation, birth weight, and number of live male and female pups were determined for each litter.

In the third treatment group, within 12 h of delivery, neonates from vehicle or THC-treated dams were cross-fostered with untreated dams who had recently delivered a litter. After weaning the offspring were housed two per cage until sexual maturation. At 45 days of age, the female offspring (n = 12/treatment group) were subjected to daily vaginal lavages in order to determine their estrous cycling pattern. After two weeks, half of the females (n = 6/treatment group) were ovariectomized and administered estradiol benzoate (1 μg, sc) 38 and 14 h before testing and progesterone (500 μg, sc) 4 h before testing for lordosis behavior according to the methodology of Hardy & Debold (1971). The lordosis quotient (LQ) measured in sexually naive female rats treated with this hormone treatment regimen was approximately 50% (unpublished observations) allowing an increase or decrease in copulatory behavior parameters to be measured. Briefly, beginning at approximately 30 min after lights out, proven breeder male rats were placed with females and allowed to mount the female ten times. The latency to first lordotic posture after introduction of the male and the number of lordotic responses in ten mounts by the male (LQ) were measured for each animal. Copulatory behavior was also measured in intact male offspring at 70 days of age as described earlier (Murphy *et al.*, 1994). Briefly, ovariectomized, steroid-primed rats, used exclusively for testing male behavior, were placed with the male offspring and the following parameters were determined: latency to first mount (ML), latency to first intromission (IL), latency to ejaculation (EL), latency to first intromission post-ejaculation (PEI) and percent of animals ejaculating (%E). At 75 days of age, the male offspring were briefly anesthetized and decapitated in order to obtain truck blood for hormone analyses; female offspring were sacrificed on a diestrous day at approximately 75 days of age. Thymus and spleen from intact male and female offspring and testes from male offspring were excised, cleaned

of fat, and weighed. Thymic and spleen lymphocytes were prepared according to the method of Grossman *et al.* (1982) with some modifications. Tissues were teased apart and gently passed through a 40-μm stainless steel mesh screen to remove connective tissue. After two washes with cold PBS, cell viability was determined using trypan blue and the thymocyte cell suspension was counted. Spleen cell suspensions were centrifuged over a Ficoll-Histopaque density gradient (Sigma) and washed with PBS to isolate mononuclear cells for counting.

Plasma LH, prolactin and testosterone concentrations were determined in single radioimmunoassays using assay procedures described in our previous publications (Murphy & Tyrey, 1986; Murphy *et al.*, 1991; Chandrashekar *et al.*, 1987). The assay sensitivities were 0.024, 0.0195 and 0.025 ng/ml and the intraassay coefficient of variation was 6.1, 5.2 and 3.8% for LH, prolactin and testosterone, respectively. Gonadotropin results were expressed in terms of the NIADDK rat LH or prolactin reference preparation RP-3. Differences in mean values between groups were determined by analysis of variance followed by Neuman-Keuls post hoc test. Comparisons between only two means within an experiment were evaluated using the Student's *t*-test. Copulatory behavior parameters in control *vs* THC-exposed animals were evaluated nonparametrically with Fisher's exact probability test. In all cases, $P<0.05$ was taken as the level of significance.

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