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Perinatal or Adult Exposure to Cannabinoids Alters Male Reproductive Functions in Mice

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DALTERIO, S. L. *Perinatal or adult exposure to cannabinoids alters male reproductive functions in mice.* PHARMAC. BIOCHEM. BEHAV. 12(1) 143–153, 1980.—Oral administration of THC or CBN at a dose of 50 mg/kg body weight to pregnant and lactating female mice results in long-term effects on their male offspring, including: body weight regulation, pituitary-gonadal function, responsivity to exteroceptive stimuli from conspecifics and copulatory activity. Effects of perinatal exposure to cannabinoids on the male reproductive system did not become evident until after weaning (21 days of age). Male mice exposed to THC had reduced testes weight and elevated plasma LH levels during and after sexual maturation. In contrast, CBN-exposed males had reduced levels of testosterone (T) and LH during the prepubertal period and normal levels of these hormones after sexual maturation, although plasma FSH levels appeared reduced. In prepubertal males, production of androgen-dependent urinary pheromones, as assessed by uterine weight gain in cohabitant immature females, was not differentially affected by perinatal cannabinoid exposure. However, the pattern of body weight gain in the maturing males, the weights of the accessory reproductive organs and pituitary LH release were affected by the interaction of perinatal drug exposure and housing with an immature female. Plasma levels of T were elevated in all prepubertal males housed with an immature female for one week, whether or not the animals were previously exposed to cannabinoids. Copulatory behavior was reduced in adult males exposed to either THC or CBN during the perinatal period of sexual differentiation. Chronic treatment of adult males with 50 mg THC/kg body weight for 3 weeks increased testes weight and decreased the weights of the seminal vesicles. However, these effects were no longer evident after 7 weeks of THC treatment. The levels of copulatory activity displayed by the THC-exposed males were reduced after both 2.5 and 6 weeks of treatment. In contrast, oral administration of 50 mg/kg cannabinol for 3 weeks decreased testes and seminal vesicle weights and plasma T and LH levels. In addition, 2.5 weeks after the onset of CBN treatment, copulatory behavior was significantly suppressed. These findings indicate that both psychoactive and non-psychoactive constituents of mariuana affect pituitary-gonadal function in adult mice, and that the development of the male reproductive system is significantly altered in animals exposured to cannabinoids during critical periods of sexual differentiation. Moreover, some of the observed effects on male reproductive function and androgen-dependent behaviors may be secondary to alterations in the endocrine system produced by non-psychoactive and psychoactive components of marihuana.

Sexual differentiation Oral Δ^9-THC and CBN Transfer of cannabinoids via milk Perinatal exposure Testosterone Luteinizing hormone Follicle-stimulating hormone Pituitary-gonadal axis Pheromones Body weight regulation Sexual behavior Exteroceptive stimulus from conspecifics Testis function

MARIHUANA, and its main psychoactive component Δ^9-tetrahydrocannabinol (THC), have been reported to exert a wide range of effects on male reproductive functions. Kolodny *et al.* [47] reported that chronic marihuana use by men is associated with decreased peripheral testosterone (T) levels, reduction in sperm count, and impotence. Even though some of these findings have been disputed [58], other investigators have observed reductions in plasma T levels [46] and impaired spermatogenesis [34] in human chronic marihuana users. A reduction in plasma T, LH, ACTH, growth hormone (GH) and prolactin (PRL) levels was demonstrated in the rat after administration of cannabinoids [18, 44, 48, 80] and THC has also been demonstrated to reduce plasma T levels in the male rhesus monkey [77]. Chronic administration of cannabis extract has also been shown to suppress spermatogenesis in the mouse [25]. We have previously demonstrated that acute exposure to THC reduces plasma levels of T, LH, and FSH in mice [22].

Although it is evident that cannabinoids are capable of interfering with the endocrine and spermatogenic function of the testis, the mechanism of their action has not been established. A simultaneous decline in plasma LH and T levels observed in some studies [46,80] strongly suggests that marihuana depresses the release of LH with a resulting decline in testicular steroidogenesis. A possibility that cannabinoids inhibit the biosynthsis of T by direct action on the testis is consistent with their retention in testicular tissue [35], and by the finding that the addition of THC and other cannabinoids to incubated testicular slices or decapsulated testes interferes with the incorporation of labeled precursors into nucleic acids, lipids, and proteins [37] and with T production in vitro [21].

In addition to its reported ability to suppress homone production by the testis and the pituitary, marihuana can suppress male copulatory behavior. Treatment of male mice with cannabis resin resulted in a significant reduction in the number of mounts and attempted mounts [20]. Treatment with THC increased the latency to mount, ejaculate, and

remount by the male rat [60]. We recently reported that treatment of adult male mice with a single oral dose of THC, but not cannabinol (CBN), resulted in a complete suppression of copulatory activity. However, treatment with THC did not eliminate certain pre-copulatory behaviors such as genital sniffing and grooming in this experiment [22].

On the basis of these findings, it can be concluded that cannabinoids affect reproductive function and androgen-dependent behaviors in adult males of several species. However, the possible implications for male fetal development have not been examined. Human fetuses could be exposed to cannabinoids via placental transfer from the mother. After administration of cannabinoids to experimental animals [29, 30, 36, 41], detectable amounts of these substances have been observed in the mitochondrial fraction of the fetal brain in the dog [54], and in a variety of fetal tissues in the mouse [41]. Newborn mammals may be exposed to cannabinoids through the milk. Accumulation of labelled THC in the milk was reported in the ewe [38] and radioactivity was detected in suckling rat pups [39] after treatment of the lactating female with ^{14}C-THC. Thus, pituitary and testicular function in the fetus could be affected by maternal exposure to cannabinoids during critical periods of sexual differentiation. In rodents, the testis is reported to produce increasing amounts of androgen during perinatal periods of sexual differentiation [13]. In the rat, the peak in fetal T levels on Day 18.5 was coincident with the stabilization of the internal male reproductive structures[84]. In the mouse, T secretion by fetal mouse testes increases from Day 14 to Day 18, and is controlled by gonadotropins produced by the fetal pituitary.

A preliminary experiment was conducted to determine whether cannabinoids were transferred via the milk in lactating mice. The next question addressed in the present experiments was whether the perinatal exposure to THC, the main psychoactive constituent, or to CBN, a non-psychoactive component of marihuana affects the development of the male reproductive system. To obtain this information, we examined male offspring of female mice treated with either THC or CBN during late pregnancy and early lactation, at weaning, during puberty, and as adults with respect to several morphological, hormonal and behavioral parameters of male reproductive function. In addition, we attempted to assess pheromone production in prepubertal males exposed to THC or CBN. Uterine weight gain in immature female mice can be accelerated by exposure to male urinary pheromones [51,52] and production of these pheromones is androgen-dependent and responsive to changes in gonadal function [15].

We also determined the effects of cannabinoid-induced alterations in hormonal status during critical periods of sexual differentiation on later adult behavior. Thus, copulatory activity in adult mice exposed to THC or CBN perinatally was assessed. Hormonal status during the perinatal period determines the probability that an animal will develop the sexual behavioral repertoire appropriate to its sex [3, 4, 7, 16, 33, 40].

The second series of experiments examined the effects of repeated exposure of adult male mice to THC or CBN. The weight of the testes and other androgen target tissues, peripheral hormone levels and sexual activity were measured.

METHOD

Animals

Random-bred mice were obtained from the colony maintained at the Worchester Foundation for Experimental Biology. Animals were maintained on a 14 hr L:10 hr D lighting schedule and provided access to Purina Mouse Breeder Chow and tap water ad lib.

TABLE 1

NUMBER OF MALE MICE EXPOSED TO THC OR CBN DURING THE PERINATAL PERIOD AND EXAMINED AT WEANING, DURING PUBERTY, OR AS ADULTS

Treatment	Ages 21 days	35–37 days	60–80 days	Total
THC	20	15	17	52
CBN	20	17	20	57
Oil	20	13	28	61

Procedure

For studies on the effects of perinatal exposure to cannabinoids, adult primiparous female mice were housed with an adult male and checked daily for the presence of a copulatory plug (designated as Day 1 of pregnancy). Approximately 24 hours prior to parturition (Day 20), THC or CBN, at 50 mg/kg body weight in 20 μl sesame oil, or sesame oil alone, was administered orally. The second dose of cannabinoid or oil was administered on the day of parturition and treatment was continued daily for six days post-partum. On the day of parturition, litters were culled to 6 male pups. These males were weaned at 21 days of age and housed with their male littermates. The number of males used in each phase of the perinatal studies is provided in Table 1.

In the chronic studies on adult males, the animals were matched by litter and body weight and data were analyzed using Friedman's two-way analysis of variance for matched samples [76]. To avoid confusing the effects of female-related stimuli, ony half of the males in each treatment condition were tested for copulatory behavior

Biochemical Determinations

Blood was collected by cardiac puncture under light ether anesthesia during the middle of the light period. The concentration of T in the plasma was determined by radioimmunoassay (RIA) without chromatography [1,6], since the concentration of DHT in the plasma represents a small percent of the T level in the maturing or adult mouse. Plasma LH and FSH were measured using the NIAMDD kits for rat LH and FSH, which have already been validated for measuring mouse gonadotropins [8].

Behavioral Testing

Males were housed individually for approximately four hours prior to the introduction of a stimulus female. The stimulus females were ovariectomized and brought into behavioral estrus with 25 μg estradiol benzoate per day by SC injection for two days, and a single 500 μg SC injection of progesterone 24 hours later. The females were used for behavioral testing 7 hours after receiving progesterone [26]; they were introduced into the home cage of the male and the cage cover was replaced with a clear plastic lid to facilitate observations. The following test measures (adapted from

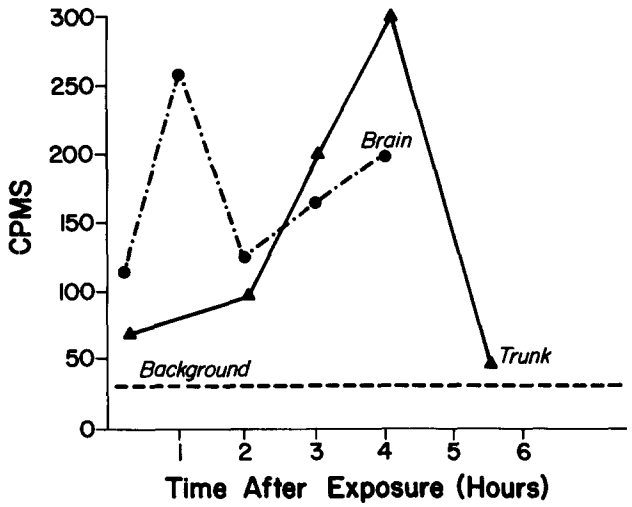

FIG. 1. Appearance of radioactivity in neonatal suckling mice after oral administration of ^{14}C-THC to the mother. The female received 2.5 μCi of ^{14}C-THC; the pups were removed at intervals indicated; radioactivity was determined separately in the trunk and in the brain.

[57]) were recorded during a one-hour test session: (1) mount latency—time from the introducion of the female to the first mount, with or without intromission by the male. (2) intromission latency—time from introduction of the female to the first mount with intromission, (3) ejaculatory latency—time from the first intromission to the beginning of the ejaculatory reflex. The number of mounts and intromissions were also recorded. The latency for an animal not exhibiting behavior was considered as 60 minutes for statistical purposes.

Transfer of Cannabinoids via Milk

A single oral dose of a mixture of ^{14}C-THC (50 mg THC/kg body weight, total radioactivity 2.7 μCi) was orally administered to lactating female mice three days postpartum. Groups of pups were sacrificed at 15 min and 1, 2, 3, 4 or 5 hours after treatment of the mother. Radioactivity in the brain and trunk was determined separately. The neonatal tissue was freeze-dried and oxidized in a Packard Tri-Carb Oxidizer and total radioactivity determined by liquid scintillation counting.

In additional groups of lactating females, the young were removed and the mice treated in an identical fashion with either ^{14}C-THC or ^{14}C-CBN. After 3.5 to 4.5 hours CBN, each female received a single IP injection of 0.1 IU oxytocin and, beginning 10 minutes later, milk was collected manually into capillary tubes [28].

Data Analysis

Data were first analyzed by analysis of variance (ANOVA) to establish the significance of any main effects and their interaction unless indicated otherwise (Fig. 2). Significant F-ratios were further evaluated by Duncan's multiple range test for paired comparisons [76,86].

RESULTS

Transfer of Cannabinoids via Milk

Administration of ^{14}C-THC to lactating females led to the accumulation of detectable levels of radioactivity in the neonate with peak levels observed at approximately four hours in the body of the neonate and somewhat earlier in the neonatal brain (Fig. 1). For both THC and CBN total radioactivity recovered from milk during the period examined was quite low, approximately 1%. Chromatography of the milk extract obtained in the second group of females revealed that 90% of the radioactivity was associated with unmetabolized THC and the rest with other compounds. The lower recovery of radioactivity after the administration of ^{14}C-CBN did not permit chromatographic identification of compounds recovered.

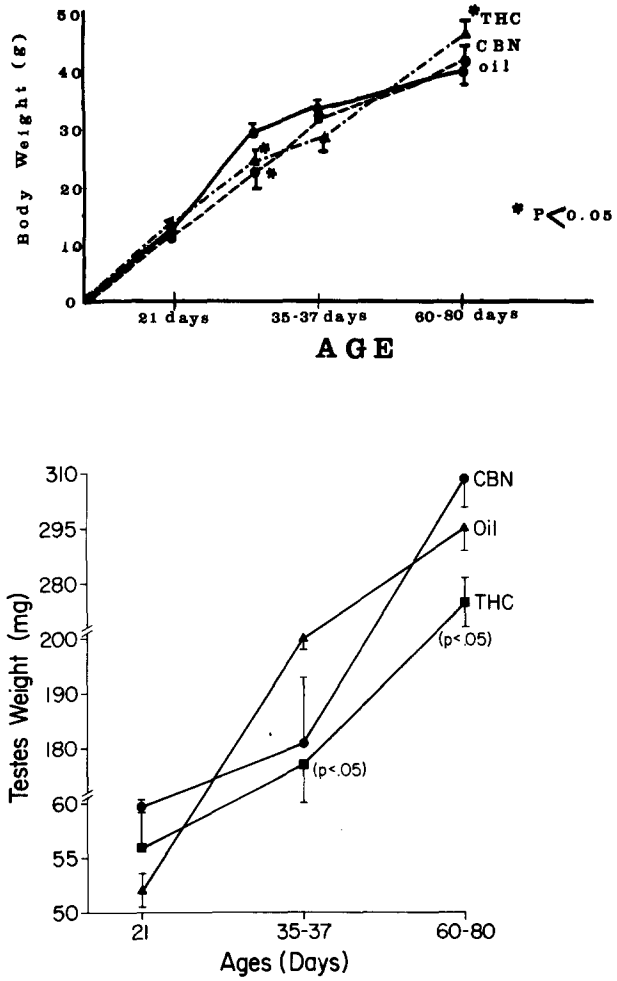

FIG. 2. Effects of treatment of female mice with THC or CBN on body weight (top) and testes weights (bottom) in their male offspring. Female mice received 50 mg/kg THC or CBN one day prior to parturition and for six days postpartum. Male offspring were examined at 21 days (weaning), 35–37 days (prepuberty) and in adulthood (60–80 days). For body weight during the prepubertal period, only those mice maintained in all-male groups are represented. (Means ± S.E.)

Perinatal Exposure to THC or CBN–Morphological and Hormonal Effects

Weaning age. Adminstration of cannabinoids to female mice just prior to parturition and for six days postpartum,

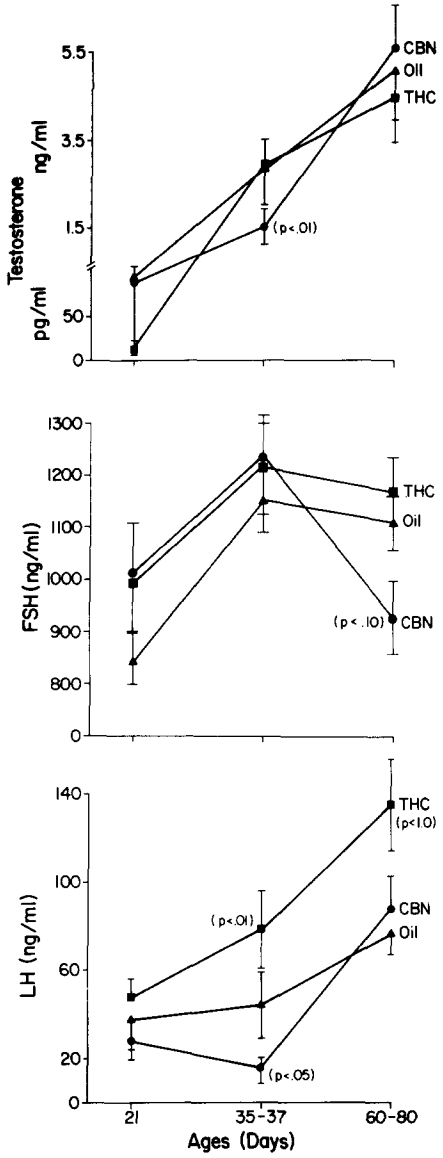

FIG. 3. Effects of treatment of female mice with THC or CBN on plasma levels of testosterone (top), FSH (middle) and LH (bottom), in their male offspring. Female mice received 50 mg/kg THC or CBN one day prior to parturition and for six days postpartum. Male offspring were examined at 21 days (weaning), 35–37 days (prepuberty) and in adulthood (60–80 days). During the prepubertal period only those mice maintained in all male groups are represented for each hormone. (Means ± S.E.) Statistical analysis of plasma hormone levels employed the Kruskal-Wallis one-way analysis of variance and the Mann-Whitney U-test [76].

produced no significant changes in body weight, testes weight, plasma levels of T, LH and FSH in their male offspring at 21 days of age (Figs. 2 and 3).

Prepubertal period. Perinatal THC exposure resulted in reduced body weight (30 days) and testes weight in prepubertal males (35–37 days) (Fig. 2). These mice also had a significant elevation in plasma LH levels, although plasma T and FSH were not affected (Fig. 3). In contrast, prepubertal males which had been previously exposed to CBN had reduced concentrations of plasma LH and T, with no difference in FSH levels (Fig. 3). Although testes weights (35–37 days) were not significantly reduced by CBN exposure, at 30 days of age body weight was depressed (Fig. 2). Perinatal cannabinoid exposure did not affect the weights of full or empty seminal vesicles in 35-37 day old mice (Fig. 5).

TABLE 2

UTERINE WEIGHTS IN IMMATURE FEMALE MICE (28 DAYS OF AGE) HOUSED FOR ONE WEEK WITH 30-DAY-OLD MALE MICE PERINATALLY EXPOSED TO Δ^9-TETRAHYDROCANNABINOL (THC) OR CANNABINOL (CBN)
(MEANS ± SE)

(n)	Oil (7)	THC (7)	CBN (7)
Body wt. (g)	20.3 ± 0.49	20.5 ± 0.55	19.0 ± 0.38
Uterine wt. (mg)	92 ± 3.7	104 ± 8.3	98 ± 12.0

No significant ($p<0.05$) difference between groups.

Adulthood. As was the case with the prepubertal males, adult 60–80 day old mice exposed to THC had a reduction in testes weights (Fig. 2) and an elevation in peripheral LH concentrations (Fig. 3). Apparent reductions in seminal vesicles weights (256 ± 14 vs 278 ± 13 mg) and in plasma T levels were not significant (Fig. 3) and there were no changes in plasma FSH levels. However, body weight was significantly greater in THC-exposed mice than in controls (Fig. 2).

In contrast, the mice exposed to CBN were not different from control males with respect to any of these parameter when examined at 60–80 days of age (Figs. 2 and 3). Only the apparent decrease in plasma FSH levels (917 ± 70 vs 1101 ± 54) approached statistical significance ($p<0.10$).

Perinatal Exposure to THC or CBN — Responsivity to Female Conspecifics

Prepubertal period. Perinatal drug exposure did not affect urinary pheromone production, as indicated by uterine weight gain in immature female mice (Table 2). However, the males housed with a 21-day-old female for one week differed from their all male group-housed littermates on several parameters, $F(3,39) = 3.8$, $p<0.05$. Body weights were higher in males housed with each other than in those mice exposed to the immature female. There was a significant interaction, $F(1,39) = 3.2$, $p<0.05$, between housing condition and drug treatment for adrenal weights. Thus, adrenal weights were significantly higher in THC-exposed mice housed with a female than in THC males housed together (Fig. 4). The opposite was true for the control males. All CBN-exposed males, regardless of housing, had adrenal weights comparable to the weights observed in group-housed control and female-exposed THC mice.

Seminal vesicles weights in control and CBN-exposed males were significantly affected by housing condition. In the control group, seminal vesicles with secretions were heavier in males housed with a female than in group-housed males. In contrast, CBN-exposed males exhibited a considerable reduction in seminal vesicles weights if housed with a female (Fig. 5). The pattern of weights of empty seminal vesicles was similar; however this effect was significant only in control males (Fig. 5).

Testosterone levels were elevated after housing with an immature female in all males, regardless of perinatal treat-

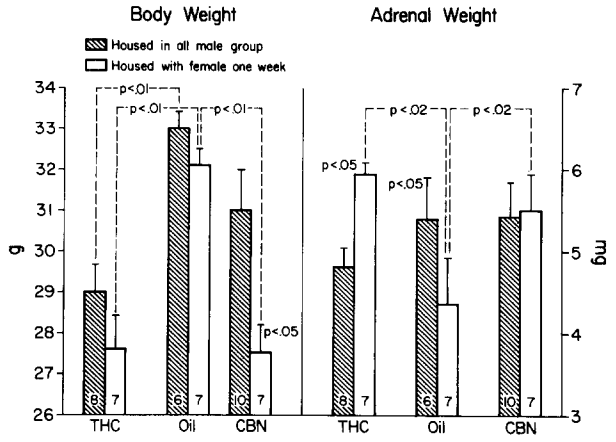

FIG.4. Effects of cannabinoids and female exposure on body weights and adrenal weights in prepubertal male mice. THC or CBN (50 mg/kg/day) was administered to females on the last day of pregnancy and during the first six days of lactation. Beginning at 30 days of age some male offspring were individually housed with an immature female, while the rest of the animals remained in all-male groups. Animals were killed between 35 and 37 days of age. (Vertical bars represent S.E.; number of animals per group is given at the base of each bar.)

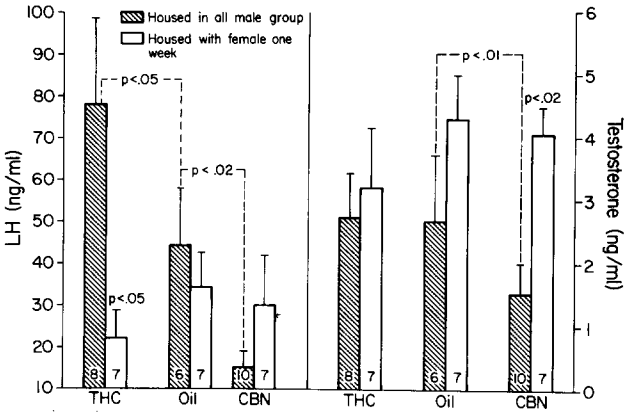

FIG. 6. Effects of cannabinoids and female exposure on plasma testosterone and LH in prepubertal male mice. For explanations, see Fig. 4. (Means ± S.E.)

ment condition, (Fig. 6). However, the THC-exposed animals were least responsive to this female-related stimulation while the CBN-exposed males housed with a female exhibited a dramatic increase in plasma T ($p<0.02$) compared to group-housed CBN males (Fig.6).

The analysis of data on plasma LH levels revealed a significant interaction ($F = 4.4$, $p<0.05$) between housing condition and cannabinoid exposure. Housing with a female brought the elevated LH levels of THC-males and the suppressed LH levels in CBN animals to levels comparable to that of control mice (Fig. 5). Testes weights in THC mice exposed to a female, were reduced (175 ± 5 vs 198 ± 9; $p<0.05$) while those in CBN-males were comparable to controls (186 ± 6 vs 198 ± 9 m). Plasma FSH levels were not influenced by perinatal cannabinoid exposure, nor housing with an immature female (1320 ± 94 oil; 1149 ± 143 THC and 1198 ± 95 CBN ng/ml).

Adulthood. Copuatory behavior in adult male mice which

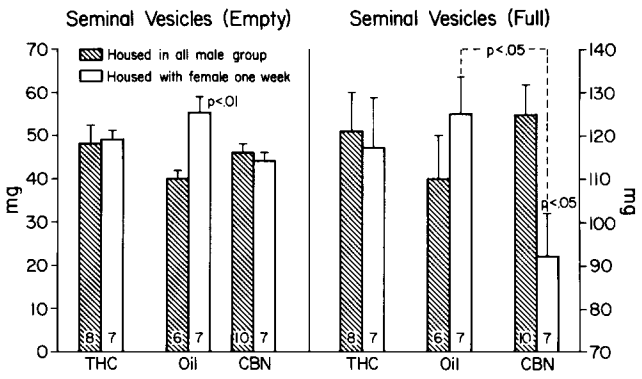

FIG. 5. Effects of cannabinoids and female exposure on the weights of seminal vesicles, with secretions or emptied, in prepubertal male mice. Detailed explanations in Fig. 4. (Means ± S.E.)

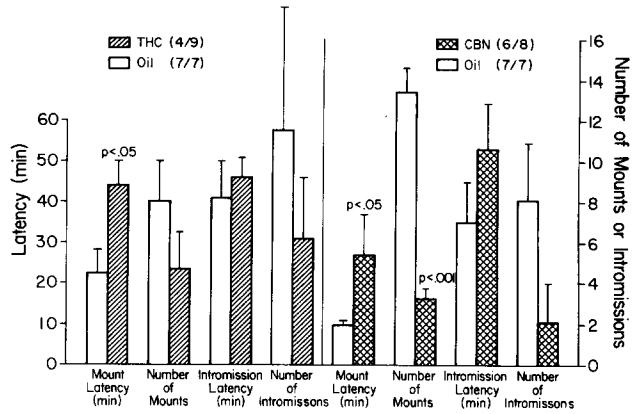

FIG. 7. Adult copulatory behavior in male mice which were perinatally exposed to THC or CBN. THC and CBN (50 mg/kg/day) were administered to female mice on the last day of pregnancy and during the first 6 days of lactation. Male offspring were housed with their male littermates since weaning and exposed to a sexually receptive female for one hour. (Proportion of males mounting.) (Means ± S.E.)

had been perinatally exposed to THC or CBN was significantly suppressed (Fig. 7). Less than half of the THC males attempted to mount a sexually receptive female ($\chi^2 = 5.65$, $p<0.02$), whereas all the control males mounted. Perinatal exposure to THC significantly increased the latency to mount ($t = 5.65$, $p<0.05$) and tended to decrease copulatory activity across the parameters measured. Exposure to CBN increased the latency to mount ($t = 2.34$, $p<0.05$) and decreased the number of mounts ($t = 4.58$, $p<0.001$).

None of the males ejaculated during the one hour test session. However, it has been reported that sexually naive male mice often require a longer period to exhibit the complete copulatory pattern [16,57].

Chronic Studies in Adult Males

There were no differences in organ weights and plasma hormone levels in males tested for copulatory behavior, and therefore the data from tested and non-tested mice were combined for statistical analysis.

THC — three weeks. Treatment of adult male mice with 50 mg THC/kg body weight three times per week for three

TABLE 3

EFFECTS OF ORAL TREATMENT WITH 50 mg Δ^9-TETRAHYDROCANNABINOL (THC)/kg BODY WEIGHT FOR THREE WEEKS (3 TIMES/WEEK) IN ADULT MALE MICE (MEANS ± SE)

	n	Oil	THC
Body weight (g)	30	37.1 ± 7	35.0 ± 0.6
Testes (mg)	30	259 ± 7	279 ± 8*
Seminal vesicles (full) (mg)	30	306 ± 10	275 ± 10*
Plasma T (ng/ml)	15	7.8 ± 2.4	5.4 ± 2.3
Plasma LH (ng/ml)	15	33.1 ± 3.2	34.3 ± 7.1
Plasma FSH (ng/ml)	15	1136 ± 98	1060 ± 89
Plasma PRL (ng/ml)	14	54.2 ± 7.4	60.2 ± 8.4
Plasma GH (ng/ml)	14	47.6 ± 7.6	41.5 ± 4.3

*Significantly different from controls, $p<0.01$.

TABLE 4

COPULATORY BEHAVIOR DURING A ONE HOUR TEST SESSION IN MALE MICE TREATED WITH 50 mg Δ^9-TETRAHYDROCANNABINOL (THC) OR CANNABINOL (CBN)/kg BODY WEIGHT THREE TIMES/WEEK (MEANS ± SE)

	n	Latency to mount (min)	No. of mounts	Intromission latency (min)	No. of intromissions
Oil	7	17.0 ± 4.1	7.6 ± 1.6	26.3 ± 6.9	5.1 ± 2.8
THC (2.5 weeks)	7	41.6 ± 8.6*	1.7 ± 1.1†	53.1 ± 6.9†	1.3 ± 1.3
Oil	7	8.4 ± 2.0	14.0 ± 3.7	26.7 ± 8.0	2.7 ± 1.8
THC (6 weeks)	7	32.7 ± 7.5*	8.8 ± 4.5	48.0 ± 7.1	2.7 ± 0.7
Oil	6	7.8 ± 1.7	17.2 ± 5.4	37.8 ± 9.1	4.7 ± 2.3
CBN (2.5 weeks)	6	21.5 ± 4.8*	3.31 ± 0.8*	53.5 ± 4.9	1.7 ± 1.3

*Significantly different from controls, $p<0.05$.
†Significantly different from controls, $p<0.02$.

TABLE 5

EFFECTS OF ORAL TREATMENT WITH 50 mg Δ^9-TETRAHYDROCANNABINOL (THC)/ kg BODY WEIGHT FOR 7 WEEKS (3 TIMES/WEEK) IN ADULT MICE (MEANS ± SE)

n	Oil n=20	THC n=20
Body weight (g)	44.6 ± 1.6	43.1 ± 1.2
Testes (mg)	282 ± 8	294 ± 10
Seminal vesicles (mg) (full)	362 ± 11	348 ± 18
Seminal vesicles (mg) (empty)	64 ± 2	69 ± 3
Adrenals (mg)	4.82 ± 0.28	5.78 ± 0.35
Plasma T (ng/ml)	2.63 ± 0.89	3.10 ± 1.80
Incidence of decrease in T in paired comparison	14/20*	
Plasma LH (ng/ml)	35.6 ± 3.3	31.8 ± 3.2
Plasma FSH (ng/ml)	824 ± 59	754 ± 54

*Significantly different from controls, by Friedman two-way analysis of variance; $p<0.05$.

TABLE 6

THE EFFECTS OF CHRONIC ORAL ADMINISTRATION (3 TIMES/WEEK FOR THREE WEEKS) OF 50 mg CANNABINOL (CBN)/kg BODY WEIGHT IN ADULT MALE MICE (MEANS ± SE)

n	Oil n=17	CBN n=17
Body weight (g)	37.0 ± 1.0	35.8 ± 1.1
Testes (mg)	295 ± 12	265 ± 10†
Seminal vesicles (mg) (full)	317 ± 13	318 ± 18
Seminal vesicles (mg) (empty)	55 ± 5	60 ± 4
Adrenals (mg)	5.4 ± 0.2	5.1 ± 0.2
Plasma T (ng/ml)	4.73 ± 1.0	3.03 ± 1.1†
Plasma LH (ng/ml)	47.0 ± 4.0	36.8 ± 2.4*
Plasma FSH (ng/ml)	1005 ± 42	1024 ± 55

*Significantly different from controls, $p<0.05$.
†Significantly different from controls, $p<0.01$.

weeks increased testes weights and decreased the weight of the seminal vesicles (Table 3). The plasma levels of T, LH, FSH, PRL and GH were not altered. However, after 2.5 weeks of THC treatment there was a significant reduction in copulatory behavior, as indicated by the increase in both mount and intromission latencies, and a reduction in the number of mounts (Table 4).

THC — seven weeks. Treatment with 50 mg/kg THC for 7 weeks did not alter the weights of the testes, seminal vesicles or adrenals (Table 5). Exposure to THC for this period reduced plasma T levels in 14 out of 20 matched pairs of mice even though mean T levels in control and treated male mice did not differ. Peripheral levels of LH and FSH were unchanged. After 6 weeks of treatment with THC, there was an increase in the latency to mount (Table 4).

CBN — three weeks. Treatment with 50 mg CBN/kg body weight three times per week for three weeks decreased testes weight and plasma levels of both T and LH, while the weights of the seminal vesicles, and adrenals and peripheral FSH concentrations were not changed (Table 6). Copulatory behavior was also reduced by CBN treatment for 2.5 weeks as indicated by an increase in the latency to mount and a reduction in the number of mounts (Table 3).

DISCUSSION

The results of these experiments indicate that exposure to both psychoactive and non-psychoactive constituents of marihuana can affect male reproductive functions. Specifically, we have demonstrated that: (1) cannabinoids are transferred via the milk to neonatal mice; (2) exposure of pregnant and lactating mice to either THC or CBN can result in long-term alterations in body weight regulation, pituitary-gonadal feedback, responsivity to exteroceptive stimuli from conspecifics, and copulatory activity in their male offspring and (3) chronic treatment of adult male mice with either THC or CBN can affect weights of the testes and seminal vesicles, plasma androgen and gonadotropin levels as well as sexual behavior.

In assessing the results obtained after exposure of mice to cannabinoids during the perinatal "critical" period of sexual differentiation, it is important to consider the possibility of effects on the young secondary to changes in maternal behavior. Our unpublished observations indicate that treatment of lactating females with an identical dose of THC does not alter the amount of time spent on the nest or in pup retrieval and does not inhibit lactation performance. Deficiency in lactation was observed in THC-treated rats [42], but in the present studies the practice of culling litters to six young seemed to have assured adequate levels of nutrition, as reflected by the similarity in body weights at weaning in all treatment groups.

Cannabinoid exposure during the perinatal period affected the somatic growth of male mice after weaning. Prior to sexual maturation body weight was reduced in THC and CBN exposed males. The suppression of weight gain in these maturing males was probably due to insufficient androgen to maintain the rapid increases in weight characteristic of this period [82]. This suggestion is consistent with the reduction in plasma T levels observed in prepubertal CBN males. After sexual maturation both peripheral T levels and body weights were not altered in animals perinatally exposed to CBN. However, the substantial elevation in body weights in adult males perinatally exposed to THC is difficult to explain.

Suppression of hypothalamic-pituitary function during development may be related to cannabinoid-induced alterations in reproductive function later in life. Perinatal suppression of hypothalamic-pituitary LHRH or FSH release could explain the reduced testes weights in THC-exposed males [11]. Neonatal suppression of LH does not affect testes weight in adult mice [69]. Gonadotrophins also appear to be important for the development of testicular LH receptors [45,81]. Suppression of FSH and/or LH by neonatal estrogen treatment in male rats depresses later binding of ^{125}I-hCG to testicular receptors, resulting in a decrease in peripheral T and FSH levels but normal LH concentrations [45]. These findings may be related to the present results in that plasma T and LH levels were significantly depressed prior to sexual maturation in CBN males, although plasma FSH was comparable to controls. In CBN-exposed adults plasma T and LH were normal, while FSH levels appeared reduced ($p<0.10$).

In THC-exposed males impairment of testicular function was indicated by the significant reduction in testes weight, and a suggestive, although not statistically significant, decrease in plasma T levels and in seminal vesicles weights in the adult. Interestingly, plasma LH levels were markedly increased in these animals. Testicular LH receptors may have been altered by a direct effect of THC on the neonatal testis, permanently influencing its reponsiveness to LH stimulation. This suggestion is consistent with our previous in vitro studies [22] in which T production by decapsulated mouse testes was inhibited by the addition of THC only in the presence, but not in the absence, of gonadotropin stimulation. An effect of perinatal THC on testicular LH binding could account for the marked LH elevation concomitant with low normal plasma T levels and reduced testes weights in these males. This possibility is presently being explored.

Interference with gonadotropin action may explain several of the effects of perinatal exposure to cannabinoids on the male reproductive system. However, it may be difficult to explain the deficiencies in adult copulatory behavior observed in THC or CBN exposed males by this mechanism, since neonatal treatment with antiserum to LH or LHRH does not affect adult sexual behavior [11,69]. More sustained suppression of LHRH release by perinatal cannabinoid ex-

posure may have influenced later pituitary sensitivity to LHRH. It is also possible that interference with this releasing factor could affect sexual behavior, since it has been postulated that LHRH may act as a neurotransmitter directly affecting CNS control of sexual activity, in addition to its effects on pituitary gonadotropin release [62]. Thus, suppression of hypothalamic LHRH could possibly account for the depressed plasma LH and FSH levels and deficits in copulatory behavior in CBN-exposed males.

Cannabinoids decease gondotropins and T levels in the adult male. Testosterone has been shown to be essential for the perinatal brain dimorphism establishing a predominance of the male copulatory pattern [7,10]. In a preliminary study [23] exposure to THC or CBN from days 12 through day 16 of gestation reduced T concentrations in male fetal mice. It thus appears plausible that the perinatal exposure to THC or CBN could also have suppressed T production. This may have permanently altered hypothalamic responsiveness to later adult hormone levels [4, 55, 72] or the sensitivity of CNS and peripheral target tissue, which affect adult copulatory activity [69]. It has been reported that the neonatal organization of aggression in mice appears to be correlated with T uptake in CNS [85]. Moreover, interference with the action of testosterone early in development by stress [83], neonatal administration of estrogens [26, 43, 49], aromatase inhibitors [14], androgens [27], anti-androgens [63] or castration [69] results in alterations in adult copulatory behavior.

It has been suggested that the effects of neonatal treatment with estradiol on adult sexual behavior may be due to suppression of T production by a direct action on the neonatal testis [43,75]. Reduction of T production in fetus or neonate could also have affected the establishment of hypothalamic estradiol receptors [56], and we are presently investigating this possibility. Aromatization of T to estradiol within hypothalamic nuclei is believed to have an important role in the expression of male copulatory behavior in adult rodents [3,7]. In addition, the presence of estradiol within CNS during perinatal 'critical' periods of brain differentiation has been shown to be essential in the development of male copulatory behavior in adulthood [14]. It is not inconceivable that THC effects on sexual differentiation may be related to the role of estrogens in this process. THC was reported to inhibit aromatase activity in human placental tissue [12] and to exhibit estrogenic properties in several experimental systems [73,78]. However, the latter findings are a matter of some controversy [64]. Whether cannabinoids can interfere with aromatization of T within the hypothalamus or exhibit estrogenic actions in male mice in vivo remains to be determined.

Hormonal status may moderate the effects of cannabinoids on the CNS during critical periods of development [31, 32, 70, 71]. Recent reports on the development of rats prenatally exposed to marihuana extract [32,71] described a sex difference in the response to cannabinoids in terms of learning ability in the adult. Male, but not female, offspring of cannabinoid-exposed females were significantly inferior to controls in their performance on the Lashley III maze. Thus, interference with androgen action may be of primary importance in the mechanism of cannabinoid action during sexual differentiation. Sex differences in the response to cannabinoids have not been examined in this study.

The differential effects of housing with an immature female on the function of the pituitary-gonadal axis in cannabinoid-treated and control prepubertal males were unexpected. These findings suggest that the alterations in the endocrine system produced by perinatal exposure to THC or CBN can be modified by exposure to exteroceptive stimuli from an immature female mouse and that the responses to these stimuli are altered by cannabinoids. According to Maruniak, Coquelin and Bronson [55], male mice develop the capacity to secrete LH in response to adult female urine between 24/36 days of age, but this response, once developed, rapidly habituates. Thus, in the present study, housing with an immature female appears to normalize both the decreased plasma LH levels in the CBN-exposed males, and the increased plasma LH levels in the THC-exposed animals. However, the control animals showed no evidence of an LH-response to the presence of an immature female. Perhaps at 37 days of age this response had already habituated. The elevation in plasma T levels and the corresponding increase in seminal vesicles weights does suggest that the LH-response did occur in these control males at some point in their cohabitation with the females. In contrast, CBN-exposed males were possibly just beginning their response to the female at this age, as evidenced by a substantial increase in plasma T and LH, in the presence of reduced seminal vesicle weights. These results may suggest that there is some maturational delay in endocrine function in the cannabinoid-exposed animals.

Although we have previously reported an acute increase in plasma T levels in adult male mice exposed to an adult female [53] and acceleration of male sexual maturation in the presence of an adult female has been described [55,79], a sustained increase in peripheral T levels after exposure to a sexually immature female was not anticipated. In view of the complex interactions among the changes in various parameters of reproductive function and housing with an immature female, it is difficult to conclude that exposure to female conspecifics is capable of correcting alterations produced by perinatal exposure to THC or CBN. Some parameters did return to normal, while others did not. In addition, the deficiencies in sexual behavior observed in adult males may reflect a permanent alteration in some CNS mechanisms mediating the expression of these behaviors, and not simply a failure of the acute endocrine response to female-related stimuli.

The evidence obtained from long-term treatment of adult male mice with THC or CBN indicates that both the psychoactive and the non-psychoactive constituents of marihuana decrease sexual behavior concomitant with alteration in testicular function. Bermant and Davidson [10] indicate that measures of sexual arousal, such as latency to mount or intromit, seem most apt to correlate with plasma T concentrations. The androgen requirement for the maintenance of normal levels of copulatory behavior in the castrate appears to be quite low [17]. However, alcohol reduces both plasma T levels [59] and sexual arousal [74] in humans. Clinical studies present evidence that in hypogonadal patients receiving T replacement therapy, declining levels of peripheral androgen are observed to reduce libido, and interfere with erectile potency [50,61]. We have recently observed that copulatory activity was reduced in castrated mice given a single injection of free (non-esterified) T, even though plasma T levels in these animals remained on the higher end of the normal range [5] throughout the period of behavioral observation [24]. Since plasma T levels in these T-treated castrates were rapidly declining during testing, it is possible that a drug-induced decline in T levels may inhibit sexual behavior even though the absolute levels of T in the plasma appear normal.

In summary, our results demonstrate that exposure to both the psychoactive and the non-psychoactive constituents of marihuana during the perinatal period or in adulthood can alter later reproductive functions in male mice. Furthermore, reductions in sexual activity may be secondary to alteration in endocrine function. In spite of reservations as to the validity of making interspecies comparisons, these findings reinforce the possibility that exposure of human males to marihuana prenatally and/or during adolescence and adulthood may result in impaired reproductive and sexual function.

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