and may account for a significant fraction of all heterozygous females with extreme phenotypes.

Our interpretation of extremely unbalanced mosaic phenotypes in terms of selection for an X-linked gene is supported by various lines of evidence, including the tissue specificity of the selection process recently discussed by Migeon (*25*). A similar explanation has been proposed to explain differences in the expression of parental X chromosomes in the mule, the interspecific hybrid between horse and donkey (*26*). In the subject family it was not possible to study somatic cells other than erythrocytes and leukocytes, but the possibility of growth advantage conferred by a single gene could be tested experimentally in mixed cell cultures (*27*) that mimic the somatic cell structure of female heterozygotes who are X-chromosome mosaics (*28*).

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Perinatal Exposure to Cannabinoids Alters Male Reproductive Function in Mice

Abstract. *Oral administration of Δ^9-tetrahydrocannabinol or cannabinol to female mice late in pregnancy and during early lactation alters body weight regulation and pituitary-gonadal function and suppresses adult copulatory activity in their male offspring. These findings suggest that both psychoactive and nonpsychoactive constituents of marihuana can affect the development of male reproductive functions in mice.*

Marihuana can affect the reproductive system and androgen-dependent behavior in adult males of several species (*1*). However, the effects of cannabinoids on male sexual differentiation in the fetus have not been examined. Cannabinoids cross the placental barrier and accumulate in a wide variety of fetal tissues, including the mitochondrial fraction of the brain (*2*). Moreover, newborn mammals may be exposed to cannabinoids through milk. Labeled Δ^9-tetrahydrocannabinol (THC) accumulates in the milk of the ewe, and radioactivity can be detected in suckling rat pups after treatment of the lactating female with ^{14}C-labeled THC (*2*). Thus, it is conceivable that fetal pituitary and testicular function could be affected by maternal exposure to cannabinoids during critical periods of development. In rodents, the testis is reported to produce increasing amounts of androgen during perinatal sexual differentiation. In the mouse, this increasing testosterone production is controlled by the fetal pituitary (*3*).

Manipulation of pituitary gonadotropins or gonadal steroids during certain critical perinatal periods of sexual differentiation can alter the development of reproductive structures and sex-typical behavioral responses, including copulatory behavior (*4*). In addition, it has been reported (*5*) that prenatal exposure to cannabinoids affects learning ability in adult rats. Hormonal status may moderate these effects on central nervous system function, since male, but not female, rats prenatally exposed to cannabinoids exhibited inferior performance in a maze learning task.

We have recently reported (*6*) that perinatal exposure to THC, the main psychoactive component of marihuana, or cannabinol (CBN), a nonpsychoactive component, affects reproductive functions and body weight regulation in male mice prior to sexual maturation. The present study determined the consequences of perinatal exposure to THC or CBN on body weight, pituitary-gonadal function, and sexual behavior in adult male mice.

Adult primiparous female mice were obtained from a colony of randomly bred mice at the Worcester Foundation (*7*). They were housed with a sexually experienced male and checked daily for the appearance of a copulatory plug. The day the plug appeared was designated day 1 of pregnancy. Approximately 24 hours prior to parturition (day 20), the female received an oral dose of 50 mg of THC or CBN (50 mg per kilogram of body weight) in sesame oil (20 μl) or a dose of sesame oil alone. This dosage of THC or CBN will alter testis function in adult male mice (*7*). The second dose of cannabinoids was administered on the day of parturition, and treatment

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was continued daily thereafter for 6 days (*8*). At birth, the young were sexed and each litter was culled to six male pups. These males were weaned at 21 days of age and housed with their male siblings until maturity (60 to 80 days of age).

In adulthood, a group of mice from each perinatal treatment condition was tested for copulatory activity (*7*). One week later, blood was drawn from tested and nontested males by cardiac puncture under light ether anesthesia. The concentrations of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were determined by radioimmunoassay (*9*). Immediately after blood collection, the mice were killed by cervical dislocation and their body weights and weights of their testes and seminal vesicles were recorded. Since there were no differences between males tested for copulatory activity and those not tested, data for all males from each perinatal treatment condition were combined for statistical analysis.

Table 1 presents the effects of perinatal exposure to THC or CBN on body weight and reproductive organ weights and on concentrations of plasma testosterone, LH, and FSH. Perinatal exposure to THC increased body weight in adults while significantly decreasing testes weight. The weight of seminal vesicles appeared to be reduced, but the difference was not statistically significant. Plasma concentration of LH was elevated in THC-exposed males ($P < .01$), whereas plasma testosterone concentration appeared to be lower (although this difference was not statistically significant). Peripheral FSH concentration was unchanged.

In contrast, plasma concentrations of testosterone and LH, body weight, and weights of testes and seminal vesicles were not altered in males exposed to CBN during the perinatal period (Table 1). However, in these mice there was an apparent decrease in the concentration of plasma FSH that approached statistical significance ($P < .10$).

Copulatory behavior was significantly suppressed in adult male mice that had been perinatally exposed to THC (Table 2). Less than half of the THC males attempted to mount a sexually receptive female, whereas all control males mounted [$\chi^2_{(1)} = 5.65, P < .02$]. In addition, mount latency was increased in the THC-exposed males ($P < .05$). A similar reduction in sexual activity in the CBN-exposed males was indicated by an increase in mount latency ($P < .05$) and a reduction in the number of mounts ($P < .001$). Several CBN-exposed males ceased copulatory activity well before the termination of the test period, even though they had not ejaculated. None of the males from any group ejaculated during the 1-hour test session. However, it has been reported that sexually naïve male mice often require longer than an hour to exhibit the complete copulatory pattern (*10*).

Table 1. Effects of perinatal exposure to Δ^9-tetrahydrocannabinol or cannabinol on the reproductive system and plasma hormone concentrations in adult male mice. Data are expressed as means ± standard error; N = number of mice. For statistical evaluations of body and organ weights, a single classification one-way analysis of variance was used, with Duncan's multiple range test being used for paired comparisons. Due to the characteristics of the distributions for hormone values, the nonparametric Kruskal-Wallis one-way analysis of variance was used; the Mann-Whitney U test was used for further evaluation of significance.

Item	Oil (N = 28)	THC (N = 17)	CBN (N = 20)
Body weight (g)	40.6 ± 1.1	46.6 ± 1.2*	41.8 ± 1.4
Testes (mg)	295.0 ± 6.0	273.0 ± 9.0*	307.0 ± 8.0
Full seminal vesicles (mg)	278.0 ± 13.0	256.0 ± 14.0	280.0 ± 15.0
Plasma testosterone (ng/ml)	5.0 ± 1.0	4.4 ± 1.0	5.5 ± 1.4
Plasma LH (ng/ml)	76.4 ± 9.0	135.3 ± 19.0†	88.1 ± 15.0
Plasma FSH (ng/ml)	1101.0 ± 54.0	1166.0 ± 49.0	917.0 ± 70.0‡

*$P < .05$. †$P < .01$. ‡$P < .10$.

Table 2. Copulatory activity during a 1-hour test session in adult male mice exposed to Δ^9-tetrahydrocannabinol, cannabinol, or oil during the perinatal period. Data are expressed as mean ± standard error; N = number of mice.

Copulatory behavior	Oil* (N = 7)	THC (N = 9)	Oil* (N = 7)	CBN (N = 8)
Latency to mount (min)	22.4 ± 5.4	44.7 ± 6.5†	10.1 ± 2.0	30.9 ± 8.1†
Number of mounts	8.0 ± 2.0	4.7 ± 2.3	13.4 ± 1.3	3.2 ± 1.7‡
Intromission latency (min)	41.1 ± 8.4	46.3 ± 5.8	35.3 ± 9.4	53.6 ± 6.4
Number of intromissions	11.6 ± 6.3	6.4 ± 3.3	8.0 ± 3.5	2.1 ± 2.1
Proportion of animals mounting	7/7	4/9§	7/7	6/8

*Two groups of control males were used; each was tested in conjunction with either the THC or CBN males. †$P < .05$, Student's t-test. ‡$P < .001$, Student's t-test. §$\chi^2_{(1)} = 5.65$, $P < .02$.

These findings demonstrate that exposure to cannabinoids during the perinatal period of sexual differentiation can result in a permanent alteration in body weight regulation, pituitary-gonadal function, and copulatory activity in adult male mice. In evaluating these results, certain factors must be considered. It is possible that the effects of cannabinoids on the young mice were secondary to alterations in maternal behavior. However, treatment of lactating female mice 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 (*11*), but in the present study the practice of culling litters to six seems to have ensured adequate nutrition, as is reflected by the similarity in body weights at weaning in all groups (*7*).

The mechanism of cannabinoid action on male sexual differentiation is not well understood. It is possible that THC or CBN may interfere with LH release by the fetal pituitary [as they do in adult mice (*6*)], thereby suppressing testicular steroid production. In preliminary studies, exposure to THC or CBN during gestation (days 12 to 16) reduced fetal testosterone concentrations in mice (*12*). Interference with testicular androgen production during certain critical periods of sexual differentiation has been shown to affect development of the male reproductive structures, fertility, steroid uptake in various brain regions, target organ sensitivity to postpubertal hormone concentrations, and sex-typical behavioral responses (*5*).

The possibility of a direct effect of THC on the fetal testis must be considered in view of the evidence that cannabinoids suppress testosterone production by decapsulated mouse testes (*13*). A direct effect of cannabinoids on testicular steroidogenesis is consistent with reports that THC and CBN in vitro interfere with testicular cholesterol esterase (*14*), the enzyme responsible for providing the precursors for testosterone production. Furthermore, the results we obtained in vivo demonstrate that short-term exposure to THC can result in an accumulation of esterified cholesterol in the testis that is concomitant with a decrease in peripheral testosterone levels (*7*).

Some of the findings in this study

imply that THC and CBN may have different effects on the function of the pituitary-gonadal axis. The previously described decrease in plasma concentrations of testosterone and LH in CBN-exposed immature males (*6*) is evidence of depressed pituitary-gonadal function during prepuberty, although the concentrations of those hormones were normal in the adults. Conversely, in THC-exposed mice there was a disturbance in pituitary-gonadal feedback indicated by a marked increase in pituitary LH release in conjunction with nearly normal amounts of testosterone during prepuberty and after maturity.

It would be of interest to know whether THC interferes with the development of testicular LH receptors, thereby affecting testis responsiveness to gonadotropic stimulation. An effect of THC on LH binding is consistent with findings from our studies in vitro (*7*) that THC suppresses testosterone production in decapsulated testes only in the presence of gonadotropic stimulation.

Hypothalamic function could have been affected by perinatal exposure to THC or CBN. It has been reported (*15*) that neonatal administration of monosodium glutamate, which produces selective lesions in the arcuate nucleus, results in obesity, hypogonadism, and increased serum LH concentrations in adult rodents. A similar action of THC on the hypothalamus may explain the significant increases in body weight and plasma LH and the reduced testis weight in THC-exposed adult mice in the present study. It is also possible that exposure to THC interferes with the development of steroid receptors in the preoptic hypothalamus, the area that mediates sex-typical behavioral responses. Testosterone influences the development of hypothalamic androgen receptors. In addition testosterone, by means of its aromatization to estradiol, ensures the establishment of estrogen receptors within the central nervous system. The presence of estrogen within the hypothalamic nuclei is believed to be critical to the expression of sexual behavior in the adult male (*16*). Cannabinoid-induced interference with estrogen action in the central nervous system is consistent with recent findings that THC may inhibit aromatase enzymes and affect androgen binding in human placental tissue and with the proposal that THC has estrogenic effects (*17*).

Hypothalamic function, particularly that involving synthesis or release of luteinizing hormone–releasing hormone (LHRH), may have been affected by perinatal CBN exposure. This releasing hormone has been implicated as a neurotransmitter directly involved in regulating sexual behavior as well as pituitary gonadotropins (*18*). Interference with LHRH production may explain the reduction in plasma LH concentrations in prepubertal males perinatally exposed to CBN that we reported elsewhere (*6*). Such an effect may also be related to the apparent decrease ($P < .10$) in plasma FSH levels and to the significant reduction in copulatory behavior observed in CBN-exposed adult mice in the present study.

The results of these experiments suggest that exposure to THC or CBN during sexual differentiation may have a combination of effects on the fetal testis, pituitary, and hypothalamus. Early exposure to both the psychoactive and nonpsychoactive components of marihuana can alter reproductive function and copulatory behavior in male mice. It is conceivable that subtle, cannabinoid-induced changes in the hormonal environment during critical perinatal periods of sexual differentiation result in long-lasting alterations in male reproductive function and in the development of sexual dimorphism in the central nervous system mediating copulatory behavior.

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