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Suppression of Male Copulatory Behavior By Δ^9-THC is not Dependent on Changes in Plasma Testosterone or Hypothalamic Dopamine or Serotonin Content[1]

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SHRENKER, P. AND A. BARTKE. *Suppression of male copulatory behavior by Δ^9-THC is not dependent on changes in plasma testosterone or hypothalamic dopamine or serotonin content.* PHARMACOL BIOCHEM BEHAV 22(3) 415–420, 1985.—Castrated B6D2 F1 male mice were tested for their sexual responses after being administered 0.5 mg/kg Δ^9-tetrahydrocannabinol (THC), 50 mg/kg THC or oil. The animals that received 50 mg/kg, but not 0.5 mg/kg THC, showed deficits in copulatory behavior. Another group of B6D2 F1 castrates were given testosterone propionate (TP) replacement therapy plus 50 mg/kg THC or oil. Similarly, those mice which received 50 mg/kg THC showed behavioral deficits. Lastly, a group of intact B6D2 F1 males were treated with 0.5 mg/kg THC, 50 mg/kg THC or oil, were bled and decapitated, and their brains removed 10 min or 4 hr after treatment. Plasma testosterone (T) and hypothalamic dopamine (DA) levels were unaltered 4 hr after treatment with 50 mg/kg THC, but the concentration of serotonin (5-HT) in their hypothalami was elevated. This effect of THC on hypothalamic 5-HT concentration was not apparent in a larger group of randomly bred animals that were tested. These data strongly suggest that THC's behavioral effects are not mediated by variations in T levels, or by changes in hypothalamic 5-HT or DA concentrations.

Mice Genetics Testosterone Sexual behavior Luteinizing hormone Δ^9-tetrahydrocannabinol

THE major psychoactive component of marihuana, Δ^9-tetrahydrocannabinol (THC), and a non-psychoactive component of marihuana, cannabinol (CBN), have both developmental and temporary effects on behavior and the endocrine system [10–12, 16, 24]. In adult male mice, 4 hr after exposure to 50 mg/kg of THC, there is a dramatic decrease in plasma concentrations of testosterone (T), luteinizing hormone (LH) and follicle stimulating hormone (FSH) [14]. Similarly, plasma T concentrations in males exposed to 100 mg/kg THC are depressed, but there is no significant decline in LH or FSH [14]. Both groups are characterized by an absence of male copulatory behavior. Since the common endocrine dysfunction in both groups is depressed T levels, it appears that this may be the factor responsible for their behavioral dysfunction. This hypothesis is strengthened by results from animals exposed acutely to CBN. Unlike THC-exposed animals, CBN-exposed mice do not show a deficit in reproductive function, and do not have depressed plasma T levels 4 hr after exposure [14]. Thus, there is a positive correlation between the effects of cannabinoids on adult plasma T levels and copulatory behavior.

One purpose of this study was to determine if THC's effect on behavior is mediated by variations in T. This hypothesis was tested utilizing B6D2 F1 male mice, whose sex behavior is relatively independent of T [33]. Additionally, these experiments were designed to test other potential mechanisms of THC action on copulatory behavior. Both dopamine (DA) and serotonin (5-HT) influence the expression of male copulatory behavior. Depletion of brain 5-HT by the systemic administration of parachlorophenylalanine (PCPA) [42], a tryptophan hydroxylase inhibitor, or be selectively destroying serotonergic neurons using 5,6 or 5,7 dihydroxytryptamine (DHT) [4] results in increased sexual activity in male rats [18]. Contrary to the effects of 5-HT, stimulation of central dopaminergic receptors by injection of apormorphine results in increased sexual behavior in male rats, whereas haloperidol, a DA receptor blocker, results in a decrement in male rat sexual behavior [25]. Therefore, DA stimulates whereas 5-HT inhibits male sexual responses and THC has been reported to increase brain 5-HT levels [29] and decrease DA levels [27] in male mice. Thus, the behavioral deficits resulting from THC administration may occur

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through this drug's effects on neurotransmitters. Since the expression of sexual behavior is dependent on the integrity of hypothalamic nuclei [47], our second objective was to determine if THC produces changes in hypothalamic neurotransmitter concentration at the time at which behavioral deficits have been reported.

METHOD

Animals

Adult B6D2 F1 males (obtained by crossing C57BL/6 females with DBA/2 males) were purchased from Jackson Laboratory (Bar Harbor, ME) and housed in the animal facility of The University of Texas Health Science Center at San Antonio. The animals were maintained in a controlled environment with a 14 hr light:10 hr dark photoperiod (lights on at 6 o'clock) at a temperature of 22±2°C. Food (Wayne Breeder Blox; San Antonio, TX) and tap water were available ad lib. An additional group of random-bred mice from our colony, maintained under identical conditions, was used for hypothalamic neurotransmitter determinations. These random-bred mice were derived from Dw/Wf and YS/Ch Wf-dw inbred strains and the random-bred CD-1 stock. Heterozygosity was maintained by avoiding sibling and half-sibling matings.

Males of the B6D2 F1 genotype were chosen because of their unique behavioral response to endogenous T. In 1978, Batty [3] reported that, unlike CB F2 (Balb female × C57BL/6 male) males, the copulatory behavior of B6D2 F2 animals was not related to their T levels. Most rodents are dependent on T for the activation and maintenance of sex behavior. Castration generally results in the abolition of sexual behavior and the ability to perform sexually may be restored by administering T. B6D2 F1 males, however, do not lose their sexual responses when they are castrated [35] and adrenalectomized [46]. Therefore, their adult sex behavior is independent of T, making them ideally suited for testing the hypothesis that THC's behavioral effects are related to changes in androgen levels.

Procedure

The animals were given sexual experience by individually housing them with an ovariectomized female brought into artificial estrus with estradiol and progesterone injections [19]. The presence of a copulatory plug was used as an indicator that the male had attained the full repertoire of sexual behavior, including ejaculation. The males were then castrated and placed with an intact female for 5–10 days for additional experience.

Prior to experimental treatment, all males were pre-tested for copulatory behavior.

The males that showed sexual responses during the pre-test were randomly divided into 3 groups, and received either 50 μl sesame oil (control), 0.5 mg/kg THC in 50 μl oil or 50 mg/kg THC in 50 μl oil via oral administration 4 hr prior to behavioral testing. An addition group of sham-castrated mice was given 50 μl of oil and tested 4 hr later.

One week after behavioral testing, animals that had not received hormone replacement therapy were again administered either oil, 0.5 mg/kg THC or 50 mg/kg THC and sacrificed 4 hr later. They were decapitated and their brains quickly removed, put on dry ice, and stored frozen at −75°C until they were assayed for hypothalamic 5-HT and DA content.

Those males that did not copulate during the pre-test were given TP therapy (450 μg/injection) every other day for one week and then randomly assigned to one of two treatment groups. The mice were then given either 50 mg/kg THC or oil orally, and behavior tested 4 hr later.

An additional group of intact B6D2 F1 and randomly bred animals from our colony were given either oil, 0.5 mg/kg THC or 50 mg/kg THC. Ten min or 4 hr after treatment, B6D2 F1 males were bled by cardiac puncture under light ether anesthesia. After bleeding, they were sacrificed by decapitation and their brains quickly removed and frozen on dry ice. The plasma was later assayed for T and LH and the hypothalami were dissected from the brains and assayed for amines. The randomly bred mice were decapitated 4 hr after treatment and their brains removed for later neurotransmitter assays. The hypothalamus consisted of a 2 mm thick block of tissue extending from the rostral area of the mammillary bodies to the caudal part of the optic chiasm, and extending laterally to the hypothalamic sulci.

Hormone Assays

The T assay was developed after the method described by Bartke and co-workers [2]. The antibody was obtained from Dr B. V. Caldwell and cross-reacts 100% with DHT, but negligibly with other adrenal and testicular androgens. Samples were not subjected to chromatography, since it has been previously shown that DHT levels in the peripheral circulation of adult male mice are generally less than 10% of normal T levels. Therefore, values of T are indistinguishable whether measured with or without the chromatographic separation step [1,23].

Approximately 1000 counts of T, [1α,2α-^{3}H(N)]-OH (New England Nuclear No. NET 387, Boston, MA) was added to serum samples and controls and allowed to equilibrate prior to extraction. The efficiency of extraction ranged from 85% to 100%. After extraction, the samples were dried down under an N_2 stream and reconstituted with 1 ml anhydrous methyl alcohol. Because variability of peripheral T levels in the adult male mouse is very large [2], two aliquots of different sizes were pipetted from reconstituted extracted samples and then dried down. If the results obtained from these "duplicates" differed from each other by more than 10% after correcting for aliquot size, the results were not used and the determination was repeated.

Approximately 10,000 counts of labeled T was added to all tubes. Next, antibody (1:12,500 dilution) was added to all tubes except three 0% binding tubes, three total counts tubes and the water blank tubes. All tubes were then vortexed and allowed to incubate overnight at 4–8°C. At the end of the incubation period, phosphate buffer saline or a dextran-coated charcoal suspension was added to the tubes. The latter was used to separate the bound from free hormone. Tubes were then vortexed and centrifuged 10 min later at 4°C and 2800 rpm. After centrifugation, 0.6 ml of the supernatant was pipetted into mini-vials containing 4.5 ml of scintillation fluid. The counts were then converted to ng/ml using a statistical program developed by Dr. Rodbard [41]. All samples were run in one assay. The sensitivity and intra-assay coefficient of variation for this assay were 0.2 ng/ml and 4.2%, respectively.

Luteinizing hormone. Luteinizing hormone was measured by radioimmunoassay using the double antibody technique [29]. The assay was based on Dr. Niswender's ovine :ovine system, and has been previously validated for use in the mouse [5]. All samples were run in one assay. The sensitivity

TABLE 1

COPULATORY BEHAVIOR OF ADULT B6D2 F1 CASTRATED MALES 4 HR AFTER EXPOSURE TO THC

	Proportion mounting	Proportion intromitting	Proportion ejaculating	Mount latency (sec)	Intromission latency (sec)	Ejaculation latency (sec)	No. of mounts	No. of intromissions
Intact	6/6	6/6	6/6	118 ± 64‡	136 ± 61‡	1373 ± 449§	9 ± 3	15 ± 6
Oil	7/8	7/8	2/8	1564 ± 51†	1781 ± 768	4873 ± 479	14 ± 7	17 ± 10
THC								
0.5 mg/kg	8/8	6/8	3/8	209 ± 51	1578 ± 768	4271 ± 643	11 ± 5	12 ± 3
50.0 mg/kg	0/8*	0/8*	0/8	—	—	—	—	—

*$p<0.005$.
†Mean ± S.E.M.
‡$p<0.05$.
§$p<0.001$.

and intra-assay coefficient of variation were 10 ng/ml and 7.9%, respectively.

Neurotransmitter assay. Neurotransmitters were assayed according to the methods described by Steger, DePaolo, Asch and Silverman [45] and Dalterio, Steger, Mayfield and Bartke [16,17]. Briefly, ice-cold 0.1 N $HClO_3$ that contained 3-methoxy-4-hydroxyphenethyl alcohol and dihydroxybenzylamine as internal standards for 5-HT and DA, respectively, was added to every tissue sample. The sample was sonicated and an aliquot of this mixture centrifuged at 12,000 × g for 1 minute for the 5-HT assay. Additionally, an aliquot of this mixture was prepared for chromatography by adding 10 mg of acid-washed alumina and mixing for 15 min in an ice-water bath. Concentrations of 5-HT and DA were calculated by comparison of peak heights with those of their standards. The sensitivity and intra-assay coefficient of variation for 5-HT were 20 pg and 6.5%, respectively and the sensitivity and intra-assay coefficient of variation for DA were 25 pg and 5%, respectively.

Sexual Behavior Test

Ovariectomized females brought into behavioral estrus with estradiol and progesterone injections were used as stimulus animals. Forty-eight hr prior to testing, females were given 10 μg estradiol benzoate (dissolved in 50 μl sesame oil) SC followed in 42 hr by SC administration of 500 μg progesterone (dissolved in 50 μl sesame oil). Six to nine hr after the second injection, a female was placed in a male's home cage [21]. If, after 15 min, the male did not show any sexual response, the female was replaced with another artificially induced estrous female. This procedure was continued until the male had been presented with three females. The last female presented to the male, however, remained in his cage until the end of the test, which was 90 min after the introduction of the first female.

The behaviors recorded were mount, intromission and ejaculation latencies and the number of mounts and intromissions during the test. Only the data from those animals that attained mounts, intromissions or ejaculation were used when determining group latencies. For operational definitions of male mouse copulatory behavior, see McGill [34].

Forced Motor Behavior

To determine if THC produced deficits in motor behavior, some animals were tested for their ability to stay on a rotorod, using a method similar to that utilized by Dunham and Miya [20]. Briefly, animals were placed on a knurled aluminum rod which was rotating at approximately 30 rpm. If the animal did not fall off within 30 sec, he was scored as unaffected [20].

Statistics

The latency, frequency, endocrine and neuroendocrine data were analyzed using the Mann-Whitney U test unless otherwise noted. All groups were compared to oil controls. The Fisher's exact test was used to compare the percentage of animals in each group which attained mounts, intromissions and ejaculations and an unweighted means ANOVA was used for some of the neurotransmitter data.

RESULTS AND DISCUSSION

Males of the B6D2 F1 genotype have been reported to be behaviorally independent of T levels since, when castrated, they are still capable of mounting, intromitting and ejaculating [33]. Our data are consistent with this hypothesis, but it is also apparent that castration does have some subtle effects on male copulatory behavior in B6D2 F1 animals. Both mount and intromission latencies in castrates were extended as compared to sham-operated controls (Table 1). Additionally, only a few of the castrates showed the ejaculation reflex. These differences, however, may be due to the length of the interval between castration and testing. McGill [33] reported that these mice go through a "difficult period" within several weeks after castration, at which time they show deficits in copulatory behavior. It may be that some or all of our mice were in this "difficult period" during testing.

The behavioral effects of THC reported here are consistent with previous findings [14]. The 50 mg/kg, but not the 0.5 mg/kg THC dose resulted in a reduction in male copulatory behavior 4 hr after administration (Table 1). This reduction, however, is not associated with a concomitant suppression of T levels in these animals, since they were castrated 7–10 days prior to testing.Thus, in this model, behavior and T levels have been dissociated, which suggests that THC's behavioral effects, at least for this genotype, are not mediated by this drug's reported effect on T levels. Additionally, consistent with previous findings [15], those animals which were castrated, had T replacement and were

TABLE 2

THE CONCENTRATION (ng/ml) OF PLASMA LH AND TESTOSTERONE (T) IN ADULT B6D2 F1 MALE MICE 10 MIN AND 4 HR AFTER EXPOSURE TO THC (MEAN ± S.E.M.)

	10 Min		4 Hr	
	LH	T	LH	T
Oil	10.88 ± 0.84 (n=5)	34.01 ± 19.91 (n=5)	13.50 ± 2.68 (n=5)	6.41 ± .96 (n=5)
THC 0.5 mg/kg	16.45 ± 1.77* (n=5)	14.70 ± 6.78 (n=4)	14.38 ± 4.65 (n=4)	45.53 ± 28.04† (n=5)
THC 50 mg/kg	11.69 ± 0.85 (n=5)	17.10 ± 10.40 (n=5)	14.98 ± 5.27‡ (n=3)	7.18 ± 3.47 (n=5)

*$p<0.05$.
†$p<0.01$.
‡Two animals were discarded because of their unusually high LH values (153.19 and 515.87 ng/ml).

TABLE 3

THE CONCENTRATION (ng/mg) OF SEROTONIN (5-HT) AND DOPAMINE (DA) IN THE HYPOTHALAMI OF ADULT MALE B6D2 F1 MICE 10 MIN AND 4 HR AFTER EXPOSURE TO THC (MEAN ± S.E.M.)

			THC			
	Oil		0.5 mg/kg		50 mg/kg	
	5-HT	DA	5-HT	DA	5-HT	DA
10 min	2.14 ± 0.16 (n=5)	0.75 ± 0.14 (n=5)	2.15 ± 0.20 (n=5)	1.06 ± 0.20 (n=4)	2.49 ± 0.14 (n=3)	0.91 ± 0.08 (n=3)
4 hr	2.03 ± 0.02 (n=4)	0.95 ± 0.08 (n=4)	2.12 ± 0.02 (n=5)	1.03 ± 0.11 (n=5)	3.10 ± 0.81* (n=4)	1.10 ± 0.06 (n=5)

*$p<0.05$; Directional Mann-Whitney U test; $p<0.05$.

given THC also showed deficits in male copulatroy behavior as compared to oil controls (data not shown). Four of the six control animals mounted, intromitted and ejaculated compared to none of the seven THC-treated animals ($p<0.02$; χ^2). The deficits in male copulatory behavior cannot be attributed to deficits in motor behavior, since all animals that received 50 mg/kg THC were capable of staying on the rotorod (data not shown).

The endocrine response of intact B6D2 F1 males to THC appears to be different from that observed in other genotypes. Intact B6D2 F1 males did not show a decrease in serum T levels 4 hr after administration of 50 mg/kg THC (Table 2), but, like animals of other genotypes, they did have elevated T levels 4 hr after administration of the lower dose of THC and elevated LH levels 10 min after treatment with 0.5 mg/kg THC (Table 2). This variable endocrine response, coupled with the consistent behavioral effects across genotypes, is an additional indication that THC does not act through changes in plasma T levels to produce deficits in male copulatory behavior.

Additionally, the two oil control groups (10 min and 4 hr) appeared to differ in their T levels. Those animals bled 10 min after oil administration appeared to have higher T levels than did those animals bled 4 hr after receiving oil. This may be due to a transient stress effect produced by the oral administration of oil. Both chronic [7,28] and acute [22,30] stress have been reported to affect the pituitary-gonadal axis.

Other potential hormonal mediators of THC's behavioral effects include variations in brain estrogen levels or adrenal androgens. In regard to the former, estrogen that is produced from T in various brain regions [48,49] is believed to act directly on brain mechanisms responsible for male copulatory behavior during development [26, 48–50] and in adulthood [8,39]. Since castrate B6D2 F1 males probably have reduced levels of estrogen, particularly in the brain, variation in estrogen concentrations is unlikely to account for the behavioral effects observed. However, it has been reported that THC itself has estrogenic activity [44], although these results are controversial [37, 38, 43]. If, in fact, THC does have estrogenic activity, we might expect results opposite to those reported here, i.e., we would expect those animals receiving 50 mg/kg THC to copulate, and to copulate with more vigor than those animals that received 0.5 mg/kg THC or oil. However, the 50 mg/kg THC dose group showed no male copulatory behavior, whereas the males given the 0.5 mg/kg THC dose copulated as frequently as oil controls.

Regarding the possibility of adrenal involvement in the effect of THC, the sex behavior of B6D2 F1 males has been shown to be independent of both T levels [35] and adrenal steroids [46]. Other endocrine factors may mediate THC's effect. Both DA and 5-HT have been demonstrated to influ-

TABLE 4

THE CONCENTRATION (ng/mg) OF SEROTONIN (5-HT) OR DOPAMINE (DA) IN THE HYPOTHALAMI OF ADULT CASTRATED MALE B6D2 F1 MICE ONE WEEK AFTER BEHAVIOR TESTING

	5-HT	DA
Intact	2.11 ± 0.13 (n=6)	1.20 ± 0.11 (n=6)
Oil	2.17 ± 0.12 (n=9)	1.11 ± 0.11 (n=9)
THC 0.5 mg/kg	2.13 ± 0.11 (n=8)	1.83 ± 0.74 (n=8)
THC 50.0 mg/kg	2.02 ± 0.05 (n=8	1.18 ± 0.11 (n=8)

Animals were sacrificed 4 hr after THC treatment. (Mean ± S.E.M.)

TABLE 5

THE CONCENTRATION (ng/mg) OF SEROTONIN (5-HT) OR DOPAMINE (DA) IN THE HYPOTHALAMI OF ADULT MALE RANDOM-BRED MICE 4 HR AFTER EXPOSURE TO THC

	5-HT	DA
Oil	2.01 ± 0.09 (n=10)	0.95 ± 0.12 (n=10)
THC 0.5 mg/kg	2.10 ± 0.08 (n=11)	1.39 ± 0.22 (n=11)
THC 50.0 mg/kg	2.09 ± 0.13 (n=12)	0.94 ± 0.05 (n=12)

(Mean ± S.E.M.).

ence male copulatory behavior [32]. Dopamine has been shown to facilitate, whereas 5-HT appears to inhibit male copulatry behavior [9]. B6D2 F1 males administered THC and decapitated 4 hr later showed a slight increment in hypothalamic 5-HT concentration (Table 3). Because of the small n and the marginal increase in 5-HT seen in these animals, neurotransmitter levels were measured in additional groups of animals. B6D2 F1 males that had previously been behaviorally tested were again administered THC or oil, decapitated 4 hr later and their brains removed for neurotransmitter determinations. An additional group of randomly bred animals from our colony were given THC or oil, decapitated 4 hr later and their brains removed for neurotransmitter assay. No effects of THC on hypothalamic DA or 5-HT concentrations were found in either group (Tables 4 and 5). For the B6D2 F1 animals, tolerance to THC might account for our inability to detect variations in hypothalamic neurotransmitter concentration; however, Dalterio [10] has previously shown that deficits in male sexual behavior are still present after repeated exposure to THC over a period of several weeks. Thus, it is unlikely that the behavioral effects of THC are mediated by variations in hypothalamic DA or 5-HT concentrations. The small significant increase in 5-HT we initially found for B6D2 F1 males is most likely due to the small number of animals samples.

Although concentrations of whole hypothalamic 5-HT and DA cannot account for the behavioral deficits attributed to THC, there may still be variations in discrete hypothalamic nuclei that were masked in assays employing the whole hypothalamus. One such hypothalamic nucleus could be the medial preoptic area, which plays a major role in the expression of male sexual behavior [47]. Alternatively, THC's behavioral effect may be mediated by LHRH, which has previously been shown to stimulate male rat sexual behavior. When given intracerebrally, LHRH stimulates male sexual behavior [36], whereas a potent LHRH antagonist results in cessation of all male sexual behavior [40]. Additionally, Chakravarty *et al.* [6] and Steger *et al.* [45] reported that hypothalamic LHRH content was increased following THC administration, suggesting that less LHRH was being released to act on potential neural centers responsible for male sexual behavior. Consistent with the suggested decrease in LHRH release, Steger *et al.* [45] reported a decrease in plasma LH levels in these THC-treated animals. Although our animals do not have reduced levels of LH, the effect of THC on LH may be independent of its influence on LHRH, since exogenous LHRH sometimes fails to stimulate LH release in THC-treated animals [13]. Therefore, it is possible that our animals receiving 50 mg/kg THC had reduced release of hypothalamic LHRH, which resulted in deficits in sexual behavior. This hypothesis is currently being tested.

In summary, it appears that variations in plasma T levels and whole hypothalamic concentration of 5-HT and DA do not mediate the behavioral response to THC in the adult male mouse.

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