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Δ-9-Tetrahydrocannabinol Stimulates Receptive and Proceptive Sexual Behaviors in Female Hamsters[1]

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TURLEY, W. A., JR. AND O. R. FLOODY. *Δ-9-Tetrahydrocannabinol stimulates receptive and proceptive sexual behaviors in female hamsters.* PHARMAC. BIOCHEM. BEHAV. 14(5) 745–747, 1981.—This experiment studied the effects of Δ-9-tetrahydrocannabinol (THC) on lordosis responses and ultrasonic communication (measures of sexual receptivity and proceptivity, respectively) in female hamsters. Specifically, lordosis durations and rates of ultrasound production by estradiol-primed ovariectomized hamsters were observed following acute treatment with 1.5 mg/kg of THC, 500 μg of progesterone, or the injection vehicle. The results showed that THC can facilitate both lordosis and ultrasound production. Together with results from other laboratories, these data indicate that THC can stimulate female sexual behavior and suggest that this effect reflects a direct, nonhormonal, effect of THC on brain mechanisms for behavior.

Δ-9-Tetrahydrocannabinol Lordosis Proceptivity Receptivity Sexual behavior Ultrasounds

TETRAHYDROCANNABINOL (THC) interferes with a variety of male reproductive functions, including at least some copulatory behaviors (review in [1]). Some of these effects resemble effects of treatment with estrogen. This has led to the suggestion that THC can act as an estrogen agonist, binding to cytoplasmic estradiol receptors and thereby initiating a sequence of events similar to that stimulated by the ovarian hormone [8]. This interpretation, however, is controversial. In general, its validity is questioned by results suggesting that THC does not compete for high-affinity uterine estradiol receptors [9–10, 12]. In addition, the interpretation of THC's behavioral effects in terms of an estrogenic mechanism is questioned by results showing that changes in sexual behavior are not necessarily accompanied by reliable changes in gonadotrophin or testosterone levels [3]. Impaired copulatory behavior in THC-treated male rodents may, instead, reflect a nonhormonal mechanism, perhaps involving a general decline in activity or arousal [2].

Several of the mechanisms suggested to explain effects of THC on the sexual behavior of males predict effects on female behavior as well. However, effects of THC on the behavior of females have received relatively little attention. In an important exception, Gordon, Bromley, Gorski and Zimmermann [7] recently described the facilitation of lordosis in estrogen-primed female rats by acute treatment with THC. This effect superficially is consistent with suggestions that THC functions as a sex steroid agonist [8] or by inhibiting general activity [2]. However, Gordon *et al.* [7] argued against an estrogenic mechanism based on the failure of chronic THC treatment to affect lordosis. In addition, direct progestational effects, and effects mediated by adrenal progesterone, tentatively were ruled out because of the failure of THC to duplicate the biphasic inhibitory effect of progesterone on lordosis. Finally, high doses of THC did affect spontaneous motor activity, but these changes in activity caused decreases, not increases, in the incidence of lordosis. Gordon *et al.* [7] concluded that THC facilitates female sexual receptivity by a direct effect on the central nervous system.

The effects of THC on female reproductive behavior clearly merit additional study. For this reason, we have studied the effects of acute THC treatment on lordosis and ultrasound production by female golden hamsters (*Mesocricetus auratus*). Specifically, we hoped to determine (1) if the effect on lordosis described by Gordon *et al.* [7] could be extended to a different species; (2) if THC affects sexual proceptivity (ultrasound production, see [5]) as well as receptivity (lordosis); and, (3) if the different patterns of general activity associated with lordosis and ultrasound production [5] might be associated with different responses to THC.

METHOD

Subjects

The subjects were Lak:LVG female golden hamsters purchased from the Lakeview Hamster Colony at 56–63 days of age and first tested at approximately 200 days. Experimental

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TABLE 1
EFFECTS OF THC AND PROGESTERONE ON HAMSTER LORDOSIS AND ULTRASOUND PRODUCTION

Measure	Treatment		
	Progesterone	THC	Oil
Percentage of animals showing lordosis (N=21)	95.2‡	61.9†	0
Duration of lordosis when shown (mean sec ± SEM)*	243.2 ± 21.7‡	178.7 ± 25.4	—
Ultrasound rate (mean calls/min ± SEM)	17.0 ± 3.0‡	8.1 ± 2.2†	2.6 ± 0.9

*This comparison considered just the 13 animals that showed lordosis under both THC and progesterone.

†Different from oil, $p<0.01$ for measures of lordosis, $p<0.05$ for ultrasound rate.

‡Different from THC, $p<0.01$ for measures of lordosis, $p<0.05$ for ultrasound rate.

stimuli included sexually experienced adult males of the same strain. All animals were housed individually in 24×18×18 cm metal cages with ad lib food and water. The colony room was maintained at approximately 24–26°C and on a reversed 12:12 hr bright:dim cycle. All tests were conducted in the first half of the dim period, 4–7 hr after daily injections.

Test Procedures

Females were tested for rates of ultrasound production in clean 40×20×24 cm glass aquaria placed in a sound-attenuating chamber (Industrial Acoustics). Each test included a 15 min adaptation period, 1 min of exposure to a stimulus male, and a 2 min postmale period during which females were exposed to synthetic ultrasounds consisting of 100 msec pulses of 35 kHz sine waves repeated at a rate of 1 per 10 sec. Ultrasounds produced by test females during the 2 min postmale period were monitored using a Holgate Ultrasonic Receiver tuned to 35 kHz [5].

Each female was tested for sexual receptivity shortly after the completion of testing for ultrasound production. Specifically, females were exposed to stimulus males for 5 min periods, during which we recorded the occurrence and total duration of lordosis.

Experimental Design

Prospective subjects were ovariectomized under sodium pentobarbital (Nembutal) anesthesia, allowed at least 1 week of recovery, and then were observed for rates of ultrasound production in a single pretest following priming with estradiol benzoate and progesterone (10 μg of EB SC in 0.05 cc of peanut oil on each of the 3 days preceding that of testing; 500 μg of P in 0.10 cc of oil 5–7 hr before testing). To allow for the possibility that THC would be less effective than P in maintaining sex behavior, we selected 21 females with average call rates of at least 8/min for use as subjects. Subsequent work has shown that these animals are representative of a randomly selected population in their responses to THC and P [13].

The schedule of EB injections begun prior to the pretest was continued throughout testing. Each EB-primed female was rotated in a counterbalanced order through a series of 3 tests conducted at 4-day intervals. Each test was preceded by a different experimental treatment: (1) 1.5 mg/kg of THC SC in oil 4 hr before testing; (2) 500 μg of P SC in oil 5–7 hr before testing; or, (3) a control injection of the oil vehicle 4 hr before testing. The THC dose used here is the same as the maximally effective dose described for rats by Gordon *et al.* [7]. The use of a 4 hr interval between THC treatment and behavioral tests was based on the results of preliminary experiments [13].

RESULTS

The percentages of subjects performing lordosis following treatment with progesterone, THC or oil were compared using the Cochran Q test [11]. This analysis showed a highly significant difference in the likelihood of lordosis across the three treatments ($Q=30.9$, $p<0.001$; see Table 1). Subsequent pairwise comparisons using the same test revealed the following differences in lordosis incidence: P > THC > oil (each $Q \geq 7.0$, each $p \leq 0.01$).

Differences between THC- and progesterone-treated animals in lordosis incidence were reinforced by corresponding differences in lordosis duration (Table 1). Thus, even the 13 animals that performed lordosis following treatment with THC showed shorter lordosis durations in THC tests than they did in separate tests after treatment with progesterone ($t(12)=2.97$, $p=0.01$, t-test for dependent samples). Since most of the animals omitted from this analysis showed lordosis under progesterone but not THC, similar comparisons using all animals, or all animals that performed lordosis under any treatment, yield even more extreme differences in the same direction.

Ultrasound rates were subjected to analysis of variance with hormone or drug treatment as a repeated factor. The main effect of experimental treatment was highly significant $F(2,40) = 14.64$, $p<0.001$, see Table 1. Further examination of these data using Duncan's multiple range test [4] revealed the following pairwise differences in ultrasound rate: P > THC > oil (each $p<0.05$).

DISCUSSION

These results show that THC can stimulate lordosis and ultrasound production in estrogen-primed female hamsters. These findings extend the number of species and variety of female reproductive behaviors that can be enhanced by THC

(cf. [7]). They also raise the possibility that the sexual behaviors of different species differ in their sensitivity to THC, since a dose of THC that was comparable to progesterone in its effect on lordosis in the rat [7] was less effective than progesterone in stimulating lordosis and ultrasound production in hamsters. However, additional work using comparable injection routes and vehicles, and a greater range of THC doses, will be required to assess this possibility.

Ultrasound production and lordosis are links in a complex chain of behaviors that regulates reproduction in hamsters [5]. Aside from this similarity in function, however, the two behaviors differ clearly in motor organization and expression. Whereas lordosis is characterized by rigid immobility, ultrasonic calling often is associated with high levels of activity and apparent arousal. The ability of THC to enhance both calling and lordosis indicates not only that THC can affect different classes of sexual behavior, but also that these effects are independent of general changes in activity or arousal. In addition, subsequent work has ruled out the possible interpretation of these results as nonspecific drug effects by showing that THC stimulates sexual behavior only in females that have been primed both hormonally and socially. Specifically, we have found that: (1) females primed with EB but observed prior to contact with a male show no effect of THC on ultrasound production or lordosis [13]; and, (2) females exposed just to acute THC treatments of 2–12 mg/kg show no enhancement of sexual behavior regardless of test situation (Floody, unpublished data).

Maximal levels of ultrasound production and lordosis by female hamsters depend on estrogen and progesterone [6]. Therefore, the ability of THC to stimulate both behaviors suggests that it could be acting directly as an estradiol or progesterone agonist, or by stimulating sex steroid release from the adrenal glands. Estrogenic effects, however, seem unlikely for a variety of reasons. First, the treatment regimes with which THC has been shown to stimulate female sexual behavior are very different from the conditions under which estrogens are effective. Whereas estrogen treatment typically is completed at least 24 hr before progesterone treatment and testing, injections of THC are effective only when given after estrogen priming and shortly before testing ([7] and present results). Furthermore, stimulatory effects of THC administered in such an effective paradigm are unaffected by concurrent treatment with the potent antiestrogen CI-628 [13]. Finally, recent work has shown that THC does not compete effectively for uterine estrogen receptors in mice, rats or rhesus monkeys [9–10, 12].

THC and progesterone are similar in both the relatively rapid onset of their behavioral effects and in the dependence of these effects on prior estrogen priming. These similarities suggest that THC acts not in any estrogenic manner, but as a stimulus for adrenal progesterone release or as a progesterone agonist. The first of these possibilities has been studied relatively thoroughly, and is contraindicated by: (1) the fact that adrenalectomy caused increased, not decreased, sensitivity by ovariectomized rats to effects of THC on receptivity [7]; (2) ovariectomy in hamsters reveals little adrenal contribution to serum progesterone levels [14]; and, (3) a THC dose which is as effective as 500 μg of progesterone in stimulating lordosis in rats fails to show any biphasic inhibitory effect on lordosis such as can be produced by progesterone treatments of only 25–150 μg [7]. The last of these findings also argues against the possibility that THC acts directly as a progesterone agonist. In addition, Smith, Besch, Besch and Smith [12] studied THC binding in a mixed population of receptors from cytosols of rhesus monkey uteri and concluded that here, at least, "THC is not binding to any of the intracellular receptors for steroid hormones" (p. 326). Based on all of these considerations, we conclude that the most likely explanation for the ability of THC to facilitate sexual proceptivity and receptivity in female hamsters involves a direct, nonhormonal, effect of THC on brain mechanisms for sexual behavior.

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