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Endocannabinoid influence on partner preference in female rats

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ABSTRACT

The present study investigated the role of the endocannabinoid system on sexual motivation in the female rat. In Experiment 1, gonadally intact female rats were first tested for partner preference after a vehicle injection. Approximately 2 weeks later, all rats were tested again after an injection of the endocannabinoid antagonist, SR141716 (SR; also known as Rimonabant; 1.0 mg/kg). During the first 10 min of each partner preference test, subjects could spend time near either a male or female stimulus animal that was placed behind a wire mesh (No-Contact). During the second 10 min of each partner preference test, subjects had unrestricted access to both stimulus animals (Contact). When the female subjects were treated with SR, they made fewer visits to either stimulus animal during the no-contact phase of the partner preference test compared to when they were treated with vehicle. In Experiment 2, ovariectomized (OVX) subjects primed with estrogen were administered SR or vehicle and tested for partner preference (Experiment 2A). Approximately 2 weeks later, the subjects from the control group were tested again after an injection of SR (Experiment 2B). In contrast to Experiment 1, treatment with SR reduced the number of visits specifically to the male stimulus during the contact phase of the test in Experiment 2. Experiment 3 tested the effects of SR on general locomotion and found no effect of SR on line crossings in an open field. Finally, in Experiment 4, OVX estrogen- and progesterone-primed subjects were administered the endocannabinoid agonist anandamide (AEA: 1.0 mg/kg) or vehicle and tested for partner preference. AEA-treated subjects made more visits to the male stimulus than vehicle-treated subjects during the contact phase of the test. The results of the present study suggest that the endocannabinoid system may contribute to sexual motivation in female rats by specifically altering approach behavior.

Marijuana (*Cannabis sativa*) is the most commonly used illicit drug in the United States, with a majority of users reporting that marijuana was the first illicit drug they had ever used (SAMHSA, 2009, 2011). People report that one of the main reasons for using marijuana is to experience alterations in mood (e.g., euphoria, relaxation, and intensification of ordinary sensory experiences; (Hall et al., 2001)). The main psychoactive ingredient in *C. sativa* is delta 9-tetrahydrocannabinol (THC), which acts on endogenous CB_1 cannabinoid receptors. Of the many endogenous cannabinoids that have been discovered, anandamide (AEA) and 2-arachioldonylglycerol ether (2-AG) were the first two endocannabinoids identified and found to act as neuromodulators in the brain (Grotenhermen, 2006).

In marijuana users, activation of CB_1 receptors has been found to produce various 'aphrodisiac-like' effects (Halikas et al., 1982; Koff, 1974). However, these effects follow an inverted-u shaped dose–response on sexual behavior (e.g., performance, arousal, and desire; (Halikas et al., 1982; Koff, 1974)). Specifically, 61% of individuals who smoked approximately one joint reported an *increase* in sexual desire, whereas individuals who smoked two or more joints reported a *decrease* in sexual desire (Halikas et al., 1982; Koff, 1974). Nevertheless, there is evidence that marijuana affects men and women differently (Halikas et al., 1982; Koff, 1974). For example, after using marijuana, men report increased quality of orgasm, increased duration of intercourse, greater enjoyment of sexual activity, increased perception of partners' satisfaction of sexual activity, and greater attraction towards an unfamiliar partner as compared to when they were not using marijuana (Halikas et al., 1982; Koff, 1974). However, women report fewer of these increases in sexual motivation and if increases are reported, they are reported to a lesser degree (Halikas et al., 1982; Koff, 1974). Furthermore, in women, *increases* in sexual arousal elicited from viewing erotic stimuli are associated with *decreases* in endocannabinoid levels (i.e., AEA and 2-AG) (Klein et al., 2012). Taken together, these results suggest that activation of the endocannabinoid system facilitates sexual responses in men, but may inhibit or have limited effects on sexual responses in women.

A possible explanation for these gender differences could be an interaction between estrogen and the endocannabinoid system. Estrogen regulates the fatty acid, amide hydrolase (FAAH), which is the enzyme that degrades AEA (Deutsch and Chin, 1993; Hill et al., 2007). Downregulation of FAAH by estrogen increases AEA signaling. Although this connection between estrogen and the endocannabinoid system has not yet been explored in sexual behavior, the regulation of FAAH by estrogen

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could contribute to the inconsistent effects of cannabinoids on sexual responses in women, due to variable levels of circulating estrogen in women.

Similar to the results from human studies, studies using animal models have revealed differences in how endocannabinoids affect male and female sexual behaviors. For example, administration of AEA facilitates sexual behavior in male rats. Specifically, Canseco-Alba and Rodriguez-Manzo (2013) reported that AEA induced sexual behavior (e.g., mounts, intromissions, and successive ejaculatory series) in 50% of the male rats that previously would not copulate; an effect that persisted for at least 14 days after AEA administration (Canseco-Alba and Rodriguez-Manzo, 2013). However, Gorzalka et al. (2008) reported that antagonism of endocannabinoid signaling also facilitated sexual behavior in male rats. Specifically, administration of the CB_1 receptor antagonist AM251 produced a dose-dependent facilitation of ejaculation, such that the number of intromissions necessary to achieve ejaculation and ejaculation latency were reduced compared to rats that received vehicle (Gorzalka et al., 2008). These inconsistent results may be a function of the different populations tested in each of these two studies: non-copulating males vs. sexually vigorous males, respectively.

Administration of endocannabinoids to female rats has produced varied effects on sexual behavior. Mani et al. (2001) concluded that THC facilitated lordosis responses in ovariectomized (OVX), hormone-primed (estradiol benzoate [EB] and progesterone [P]) rats. Furthermore, intracerebroventricular infusions of the CB_1 antagonist/inverse agonist, SR141716 (SR), *inhibited* progesterone-, dopamine- and THC-facilitated sexual receptivity (i.e., lordosis responses) in female rats (Mani et al., 2001). In contrast, Lopez et al. (2009) found that treatment with the endocannabinoid antagonist/inverse agonist, AM251, significantly e*nhanced* sexual motivation, as measured by increases in lordosis ratings and proceptive behaviors (e.g., hops and darts) in EB- and P-primed OVX rats. These contradictory results prompted Zavatti et al. (2011) to examine the effects of SR on sexual motivation, receptivity, and proceptivity in female rats using the partner preference test. In their study, female rats treated with SR displayed reduced interest in both social (female) and sexual (male) stimulus animals when subjects were allowed to spend time in the vicinity of the stimulus animals confined to an area in an open field (Zavatti et al., 2011). However, SR treatment also decreased lordosis responses during a test for sexual receptivity (Zavatti et al., 2011).

Although Zavatti et al. (2011) measured some aspects of sexual motivation in a complex paradigm (i.e., partner preference), the authors did not measure all of the typical sexual behaviors displayed by the female rat. Specifically, they did not measure paced mating behavior. Although Lopez et al. (2009) tested female rats in a paced mating test, they did not record measures of paced mating behavior (e.g., latency to return to the male after receiving sexual stimulation and likelihood of leaving the male after the receipt of sexual stimulation). Furthermore, alterations in sexual receptivity confound any interpretation of alterations in the partner preference paradigm, because animals that are not sexually receptive typically do not prefer a sexual partner (Clark et al., 2004). Therefore, the present study was designed to investigate the acute influence of the endocannabinoid antagonist, SR141716 (1 mg/kg), as well as the agonist AEA (1 mg/kg) on a full range of sexual behaviors in the female rat. The partner preference paradigm used in the present study allowed us to test sexual motivation with and without physical contacts, as well as measure all typical patterns of sexual behavior in the female rat (i.e., lordosis, proceptive behaviors) and the active pacing of sexual contact by the female (i.e., paced mating behavior).

1. Method

1.1. Subjects

Fifty-two female Long-Evans rats (*Rattus norvegicus*; 200–400 g) were used as experimental subjects (Experiment 1: n = 11 sexually naïve; Experiment 2: n = 15 sexually naïve; Experiment 3: n = 14 sexually experienced; Experiment 4: n = 12 sexually experienced). Sexually experienced female Long-Evans rats (200–400 g), as well as, sexually experienced male Long-Evans rats (400–600 g) were used as stimulus animals. All rats were purchased from Harlan Sprague–Dawley (Indianapolis, IN). Rats were housed in plastic hanging cages with Aspen wood shavings used for bedding. Food and water were available ad libitum. Female rats were housed three to a cage and male rats were housed two to a cage. All rats were weighed weekly. Temperature and humidity in the animal colony were monitored daily. The lights in the colony were maintained on a reversed 12:12 h light–dark cycle (with lights off at 10:00 a.m.). All of the behavioral procedures took place during the dark phase of the cycle, under dim red light.

At least one week before any tests took place, the female subjects in Experiments 2, 3 and 4 were bilaterally ovariectomized (OVX) under Nembutal (sodium pentobarbital; 50.0 mg/kg i.p.; Henry Schein, Indianapolis, IN) anesthesia after pretreatment with atropine sulfate (2.5 mg), which reduces respiratory distress.

1.2. *Hormones* & drugs

For Experiments 1–3, the experimental female rats received either an intraperotineal (i.p.) injection of vehicle or SR (1.0 mg/kg). SR141716 was dissolved in a vehicle of 0.9% saline, TWEEN-80, and DMSO. A stock solution of 5.0 mg of SR in 500 μl DMSO and 500 μl of TWEEN-80 was sonicated for 30 min producing 5.0 mg/ml of SR. Next, the solution was diluted to 1.0 mg/ml by adding 250 μl of stock to 4.75 ml saline, producing a ratio of 1:1:8 of vehicle solutions (DMSO: TWEEN-80:Saline). For Experiment 4, the female subjects received either an injection of vehicle or AEA (1.0 mg/kg i.p.). AEA was dissolved in the same vehicle, following identical procedures used for SR. All drug injections were made 20 min prior to partner preference tests. These doses of SR and AEA were used previously and found to affect sexual behavior in rats (Canseco-Alba and Rodriguez-Manzo, 2013; Zavatti et al., 2011).

In Experiments 2 and 3, female subjects received 2.0 μg of estradiol benzoate (EB) daily at 1 p.m. for 6 days with the last dose administered 24 h prior to each behavioral test. All of the female stimulus animals and the female subjects in Experiment 4 received 10.0 μg of EB 48 h and 1.0 mg of progesterone (P) 4 h prior to each mating test. All hormone injections were administered subcutaneously in the flank. Each hormone was delivered in a sesame seed oil vehicle. The doses of EB and/or P used in the present study produce high levels of sexual receptivity in OVX rats (Blasberg and Clark, 1997). Hormones, atropine sulfate and vehicle solvents were purchased from the Sigma-Aldrich Chemical Company (St. Louis, MO). SR141716 and AEA were purchased from Tocris Bioscience (Minneapolis, MN).

1.3. Estrous cyclicity

The female subjects in Experiment 1 were monitored for one month using vaginal cytology to ensure normal estrous cyclicity. Vaginal cytology was examined once daily at 9:00 a.m., by collecting vaginal secretions using a sterile plastic pipette filled with saline (Marcondes et al., 2002). Vaginal fluid was placed onto glass slides and examined under a microscope. Female rats were recorded as being in proestrus, estrus, metestrus, or diestrus based on the proportion of cell types. Proestrus vaginal secretions consisted mainly of nucleated epithelial cells; estrus secretions consisted mainly of cornified, non-nucleated cells; metestrus secretions consisted of equal proportions of round leukocytes, cornified, and nucleated epithelial cells; and diestrus secretions consisted mostly of round leukocytes (Marcondes et al., 2002). After approximately one month of monitoring, the female subjects were tested for partner preference in the afternoon (~1:00 p.m.) of behavioral estrus. (i.e., they were in proestrus in the morning based on vaginal secretions) (Zipse et al., 2000). Immediately before drug