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Acute cannabinoid administration attenuates female socio-sexual motivation

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ABSTRACT

Endocannabinoids may normally inhibit the generation and expression of female estrous behaviors. Previous work in our laboratory demonstrated that acute administration of a CB_1 receptor antagonist (AM251) increased sexual incentive motivation in estrous female rats. The current experiment examined the effect of CP55,940, a synthetic cannabinoid agonist, on sexual motivation. Seventy-two ovariectomized female Long–Evans rats were tested for their socio-sexual motivation via a runway methodology. Baseline motivation to approach and maintain close proximity to an empty goalbox, a female conspecific, and a male conspecific was assessed over six trials. Subjects were then grouped into nine experimental conditions and re-tested for their socio-sexual motivation after one of three possible hormonal treatments and three drug doses. Hormone treatments were: oil (nonestrous), 10 μg estradiol benzoate (partially estrous), and 10 μg estradiol + 500 μg progesterone (fully estrous). Drug doses were: 0, 20, or 40 μg/kg CP55,940 (IP, 30 min prior to testing). As expected, hormonal priming with both estradiol and progesterone significantly increased sexual motivation in females that did not receive drug treatment. This occurred even though females were kept sexually-naïve throughout the experiment. CP55,940 dose-dependently attenuated sexual motivation for a male target in estrous females; the 40 μg/kg dose completely blocked sexual motivation. However, this same dose also significantly reduced social motivation for another female. Cannabinoid agonists reduce female sexual motivation, either directly by inhibiting estrus or indirectly by increasing social anxiety.

1. Introduction

There is significant evidence that both exogenous and endogenous cannabinoids can alter aspects of male and female copulatory behavior (reviewed in Gorzalka and Hill, 2006). In male rats, cannabinoid agonists tend to inhibit both appetitive and consummatory variables. Cannabinoids, including Δ^9-tetrahydrocannabinol (THC), increase mount, intromission, and ejaculation latencies and reduce intromission frequency (Ferrari et al., 2000; Gorzalka et al., 2008; Martinez-Gonzalez et al., 2004; Murphy et al., 1994). In contrast, cannabinoid antagonists can induce erections, reduce the number of intromissions necessary for ejaculation, and reduce ejaculation latencies (Castelli et al., 2007; Gorzalka et al., 2008; Melis et al., 2004, 2006; Succu et al., 2006). Cannabinoid effects on female sexual behavior tend to be less consistent. Several laboratories have shown that cannabinoid agonists can enhance receptivity in females primed with estrogen (Gordon et al., 1978; Mani et al., 2001; Turley and Floody, 1981). Mani et al. (2001) have suggested that cannabinoid-induced dopamine release may subsequently activate progesterone receptors to induce full behavioral estrus. However, under some experimental conditions and at higher doses, cannabinoid agonists significantly interfere with female sexual behaviors (Ferrari et al., 2000; Gordon et al., 1978).

The majority of work conducted on cannabinoid regulation of female sexual behavior has focused on receptivity. While the lordosis reflex is a convenient and readily measurable behavior, it is perhaps not the most externally valid choice if one's scientific goal is to model women's sexuality. Women do not experience overt changes in receptivity across the menstrual cycle and are capable of engaging in sexual intercourse at any time (Thornhill and Gangestad, 2008). However, in rats, non-human primates, and women, sexual motivation and mate preference are regulated by cyclic fluctuations in steroid hormones (Beach, 1976; Regan, 1999; Wallen, 2001). Precopulatory motivation is best assessed through methodologies that completely dissociate appetitive variables from performance (López et al., 1999), as in approach behavior tests where the subject and target incentive are prevented from physically interacting (Agmo et al., 2004). Our laboratory uses a simple, unconditioned approach methodology to assess socio-sexual motivation in both male and female rats. Subjects traverse a straight-arm runway to approach a goalbox containing a motivationally-relevant target, such as an opposite-sex conspecific. Attraction to the goalbox target is assessed by recording the amount of time that the subject spends in close proximity.

We have previously reported that AM251, a cannabinoid antagonist/reverse agonist, increases sexual motivation in estrous females tested within the runway apparatus (López et al., 2009). Pre-

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treatment with either 2 or 4 mg/kg of AM251 significantly increased the amount of time that female subjects spent near male, but not female, targets. Interestingly, this effect was only noted in females who were hormonally-primed with both estradiol and progesterone prior to behavioral testing. AM251 treatment also significantly increased the number of proceptive displays emitted by estrous females in a brief, non-paced mating test. Based on this work, we suggested that endocannabinoids may play a tonic, inhibitory role in the regulation of female estrous behavior.

The current experiment was designed to specifically assess the effect of a cannabinoid CB_1 receptor agonist, CP55,940, on female sexual motivation, across a variety of hormonal conditions. This work is a direct extension of our previous experimentation with AM251 (López et al., 2009). Based on that work, we hypothesized that CP55,940 would dose-dependently attenuate sexual motivation in estrous females.

2. Method

2.1. Subjects

A total of 76 female and 4 male Long–Evans rats (Charles River Laboratories, Wilmington, MA) were used. Female subjects were ovariectomized (OVX) at Charles River Laboratories 1 week prior to arrival at our vivarium and were given a minimum of 2 weeks post-surgery recovery time before being subjected to any experimental procedures. Subjects were approximately 70 days old at the start of behavioral testing. All females were pair-housed in plastic cages with woodchip bedding; males were individually housed in identical cages in the same room. Food and water were provided *ad libitum*. The vivarium environment was humidity and temperature controlled (~22 °C), and subjects were maintained under a reverse 12:12 light–dark schedule (lights on 2200 h–1000 h). All animals were handled daily by the experimenters for 1 week prior to the behavioral testing. The care and use of animals, and all aspects of the experimental protocol, were reviewed and approved by the campus Institutional Animal Care and Use Committee (IACUC) for compliance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

2.2. Hormones and drug

Steroid hormones were purchased from Sigma-Aldrich (St. Louis, MO). Estradiol benzoate (EB) was prepared in a sesame oil vehicle, and progesterone (P) was prepared in propylene glycol. Both hormones were injected subcutaneously at a volume of 0.1 ml. CP55,940 (Tocris Biosciences, Ellisville, MO) was prepared as in Braida et al. (2004), in a solution of cremophor, ethanol, and saline (1:1:18). Stock solution was gently sonicated before being aliquotted into three separate vials. Vials were stored at −10 °C until use, which occurred within 10 days of solution preparation.

CP55,940 was administered intraperitoneally (IP), in a volume of ~1 ml/kg, 30 min before the behavioral testing, as in previous research with this compound (e.g. Genn et al., 2004). Two doses were used: 20 μg/kg and 40 μg/kg. The lower dose was primarily chosen because it is rewarding to rats and therefore may model a human "recreational" dose (Braida et al., 2001). Moreover, 20 μg/kg does not significantly affect locomotion (Genn et al., 2004; Kosiorek et al., 2003) or increase anxiety in a social interaction test (Genn et al., 2004). After our initial trials indicated that 20 μg/kg was having a modest but non-significant effect on sexual motivation, we extended our dose analysis to include 40 μg/kg. This higher dose has been shown to decrease locomotor activity and increase anxiety under some experimental conditions (Genn et al., 2004; Kosiorek et al., 2003).

2.3. Runway apparatus

Motivational testing occurred in two identical straight-arm runways, each consisting of a wooden startbox (20×20×30 cm), a wooden alley (160×10×15 cm), and a cylindrical Plexiglas goalbox (50 cm diameter×30 cm height). Plexiglas guillotine doors separated the startbox from the alley, and the alley from the goalbox. A removable Plexiglas partition divided the goalbox arena into two semicircular halves. Thirty-five holes (1 cm diameter) drilled into the partition provided airflow between the halves. This partition allowed subjects to perceive visual, olfactory, and scent cues from the target animal, while preventing direct physical contact. Fig. 1 depicts a schematic representation of the runway apparatus.

Three infrared photocell emitter-detector sensor pairs built into each runway detected subject motion. Sensor #1 was placed 15 cm deep within the goalbox and was only triggered when the subject's entire head and body entered the goalbox. Sensor #2 was placed within the alley, 25 cm away from the goalbox door, and only became active after sensor #1 was triggered. Sensor #3 was located just outside the startbox. Sensors #1 and #2 allowed for measurement of a subject's proximity time (PT). An electronic timer started when the subject first entered the goalbox and triggered sensor #1. If the subject's entire body left the goalbox and triggered sensor #2, the timer stopped. If the subject re-entered the goalbox and triggered sensor #1, the timer would start again. This continued for a period of three minutes following the initial entry of the subject into the goalbox.

In addition to PT, we also counted subject "retreats." We defined a retreat as a complete return to the startbox after the subject had entered the goalbox. Every time the subject made a circuit between sensor #1 (goalbox) and sensor #3 (startbox), an electronic counter increased by one. Previous research has indicated that retreats can be a reflection of subject ambivalence over goalbox events and a behavioral manifestation of approach–avoidance conflict (Ettenberg, 2004, 2009). However, we primarily used retreats as a simple, nonspecific measure of subject ambulation within the apparatus, so that we could assess drug effects on locomotor capacity.

In numerous prior experiments, we have successfully used this apparatus and methodology to assess socio-sexual motivation in both male (López and Ettenberg, 2001, 2000, 2002; López et al., 1999) and female rats (López et al., 2009, 2007; Nofrey et al., 2008).

2.4. Procedure

2.4.1. Habituation and baseline phase

All runway testing took place under red-light illumination between 13:00 and 18:00 h (the middle of the subjects' active phase). It should be noted that neither subjects nor targets possessed any sexual experience prior to runway testing. This is in contrast to our most recent work on female sexual motivation (López et al., 2009). Subjects were given three habituation sessions (10 min each) within an empty runway on consecutive days, to familiarize them with the apparatus.

Baseline socio-sexual motivation of 72 female subjects was measured over the next 6 days. Each subject was tested in a nonestrous state for their motivation to maintain close proximity to one of three different goalbox targets: an adult male, an OVX (nonestrous) female, or an empty goalbox. Subjects did not receive hormone or drug treatment. Subjects ran one trial per day, and all ran for the same target on any given day. Thus, two trials per goalbox target were conducted during baseline; scores across these two trials were averaged. The day-to-day order of targets presented during baseline was randomly determined. Four OVX females and 4 intact males were used as targets.

Prior to beginning a day's trials, the assigned target (if a female or male conspecific) was confined to the goalbox for a period of 10 min to infuse the area with scent cues. The partition was then introduced

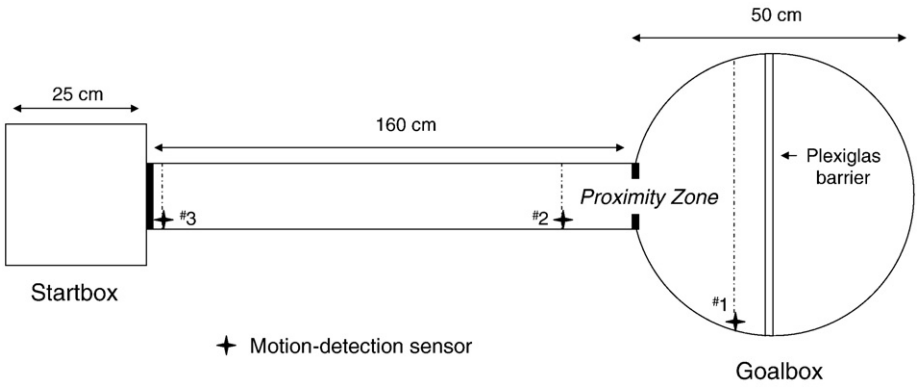

Fig. 1. A schematic of the runway apparatus used to assess socio-sexual motivation. Sensors #1 and #2 allowed for the measurement of proximity time (PT), while sensors #1 and #3 allowed for the measurement of retreats.

into the goalbox and the target placed on the side farthest from the alley. A female subject was placed into the opposite side of the goalbox for 2 min. The subject was then removed from the goalbox and immediately placed in the startbox with the door closed. After 10 s, the two guillotine doors (to startbox and goalbox) were lifted, and the subject was allowed to freely traverse the runway. The trial ended 3 min after the subject first entered the goalbox (and triggered the sensor therein). Proximity time (PT) was defined as the amount of time that the subject spent in the goalbox during this 3 min period. A higher PT indicates a more positive incentive motivation for the goalbox target. Retreats were also recorded (see Section 2.3) and used as a general measure of locomotor function. After the 3 min expired, the female subject was removed from the runway and returned to her homecage. Between individual trials, the runway was wiped down to remove any urine or feces left by the subject. The next trial was then begun; subjects were tested in the same order each day. After all of the day's trials were completed, the entire apparatus was cleaned thoroughly with a 20% ethanol solution.

2.5. Experimental phase

Following completion of the baseline phase, the subjects were divided into nine experimental groups ($n=8$/group). An attempt was made to assign subjects such that baseline PT's were approximately equal between groups. Subjects were then re-tested in the runway for their motivation to approach the same three goalbox targets: empty, OVX female, and male. Subjects were tested under one of three hormonal conditions (oil, EB-only, or EB + P) and after receiving one of three possible drug doses (0, 20, or 40 μg/kg). EB (10 μg/subject) or sesame oil vehicle was administered 48 h prior to behavioral testing. P (500 μg/subject) or propylene glycol vehicle was administered 5 h prior to the testing. CP55,940 injections (IP) occurred 30 min prior to behavioral testing.

Subjects ran a single experimental trial for each goalbox target. These trials occurred, by necessity, 4 days apart due to the induction of behavioral estrus in the EB + P subject groups. Such a treatment regimen requires that at least 3 days separate the test periods, mimicking the natural estrous cycle of the female rat. On the second day after each experimental trial, the subjects were tested in the runway for their motivation to approach an empty goalbox under nonestrous, non-drugged conditions. These trials provided subjects with a "baseline-like" experience during the experimental phase and allowed us to determine whether subject behavior was being significantly modified by successive drug treatments (possibly due to drug-environment conditioning). The experimental phase lasted a total of 11 days (with behavioral testing occurring on 6 of these days).

3. Results

Two subjects were excluded from statistical analysis: one because its experimental PT scores were more than three standard deviations from the overall mean, and one because of experimenter error. As noted, following completion of the baseline phase, an attempt was made to divide subjects into nine experimental groups with relatively equal baseline PT's. To evaluate this process, we conducted a 3 (target) × 3 (hormone) × 3 (drug) analysis of variance (ANOVA) on the baseline data once subjects were assigned to their respective groups. It should be emphasized that during baseline, there were no hormone or drug treatments; any effects linked to these two variables would therefore be indicative of nonequivalent performance. Analysis revealed a significant main effect of target ($F(2, 122)=9.12$, $p<0.001$) and a significant interaction between target and drug ($F(4, 122)=2.58$, $p=0.04$). To account for group differences in baseline motivation, all of the subjects' data were therefore converted into difference scores: experimental − baseline, for each goalbox target. A positive difference presumably reflects an increase in motivation for the goalbox target during the experimental phase.

We then conducted a series of ANOVA's on the mean difference scores of the nine experimental groups. Our primary analysis was, again, a 3 (target) × 3 (hormone) × 3 (drug) multi-factorial ANOVA ($\alpha=0.05$), with hormone and drug conditions serving as between-subject variables. Group averages are expressed as mean ± SEM. Analysis of PT differences scores indicated a significant main effect of drug ($F(2, 61)=4.98$, $p=0.01$), a hormone × drug interaction ($F(4, 61)=2.56$, $p=0.048$), and a target × drug interaction ($F(4, 122)=2.82$, $p=0.03$). We then conducted a 3 (hormone) × 3 (drug) ANOVA on the PT difference scores for each goalbox target. Fig. 2A–C presents the PT difference score means for subjects in all hormone and drug conditions, separated by goalbox target. In the empty goalbox condition, there was no significant effect of either hormonal condition or drug dose. When subjects were running for the female target, there was a significant main effect of drug ($F(2, 61)=3.40$, $p=0.04$). A post-hoc Tukey's HSD test indicated a significant difference ($p=0.03$) between the vehicle-treated subjects ($\overline{x}=11.3\pm7.3$) and those that received 40 μg/kg CP55,940 ($\overline{x}=-16.4\pm6.7$).

Analysis of the male target data revealed a main effect of hormone ($F(2, 61)=3.72$, $p=0.03$), a main effect of drug ($F(2, 61)=4.93$, $p=0.01$), and a significant hormone × drug interaction ($F(4, 61)=3.42$, $p=0.01$). Post-hoc analysis of the hormone effect with Tukey's HSD revealed a significant difference between oil-treated and EB + P subjects ($p=0.03$). Summing across drug groups, EB + P females expressed a more positive difference score ($\overline{x}=14.2\pm9.0$) than oil-treated females ($\overline{x}=-12.1\pm6.8$). Post-hoc analysis of the drug effect with Tukey's HSD revealed a significant difference ($p=0.01$) between the 0 μg/kg dose ($\overline{x}=15.7\pm7.4$) and the 40 μg/kg dose ($\overline{x}=-15.0\pm7.0$).

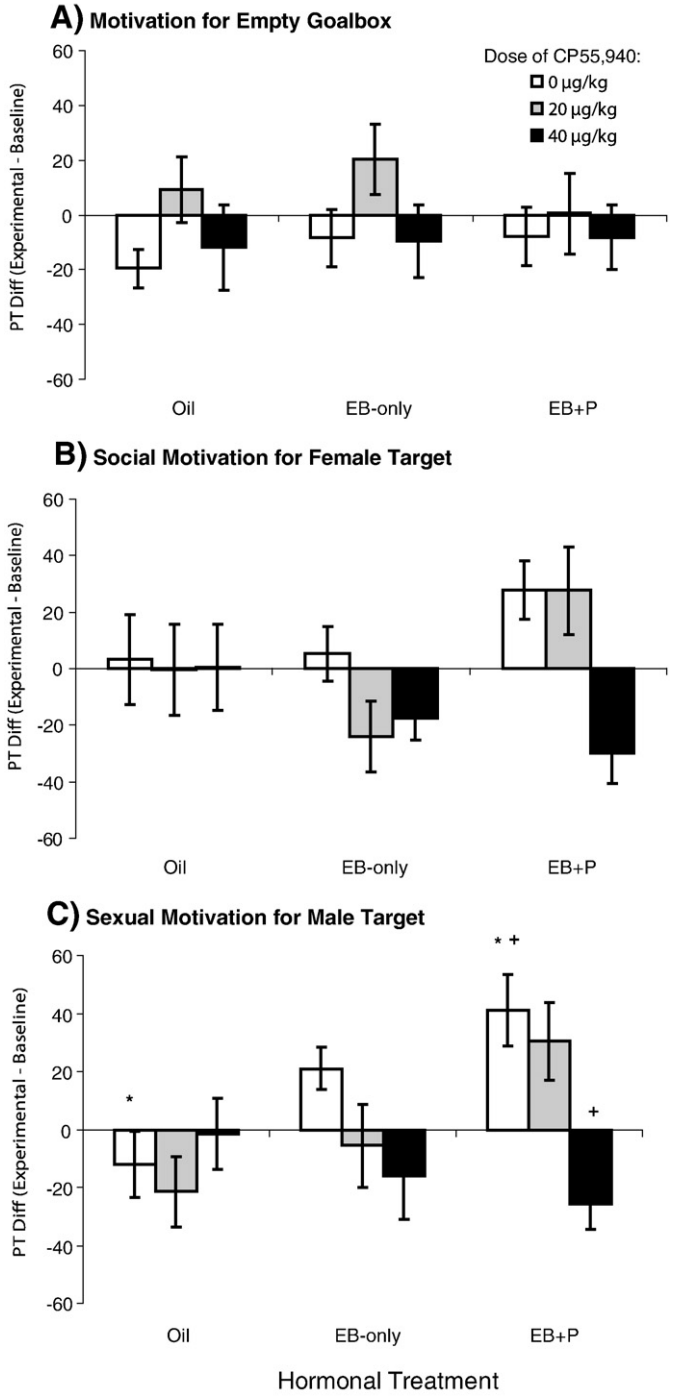

Fig. 2. (A–C). The effect of hormone and drug treatment on motivation to maintain proximity with A) an empty goalbox, B) a female target, and C) a male target in 9 groups of subjects ($n=8$/group). The dependent variable is expressed as a difference score: experimental PT − baseline PT. Hormone treatments consisted of vehicle (oil), 10 µg estradiol (EB-only), or 10 µg estradiol + 500 µg progesterone (EB + P). Drug treatments were 0, 20, or 40 µg/kg CP55,940. There was no effect of hormone or drug treatment on motivation for an empty goalbox (A). 40 µg/kg CP55,940 reduced social motivation for a female target (B). EB + P treatment significantly increased sexual motivation for a male target; this effect was completely blocked by treatment with 40 µg/kg CP55,940 (C). * and + indicate significant differences, as assessed by independent sample *t*-tests (two-tailed, $\alpha=0.0125$).

To explore the nature of the drug × hormone interaction and to test our *a priori* hypothesis that CP55,940 would reduce female sexual motivation for a male target, we conducted a small series of planned, independent sample *t*-tests (two-tailed). We compared subjects in the 0 and 40 µg/kg drug groups within each hormonal condition. Additionally, we compared the non-drugged, oil-treated control group to the non-drugged, EB + P group; this comparison allowed us to directly assess whether the hormone treatment, by itself, increased sexual motivation in our subjects. A Bonferroni adjustment was made to reduce the probability of Type 1 error across these 4 *t*-tests, giving an alpha of 0.0125.

Non-drugged, EB + P females had a statistically greater increase in proximity time (i.e. a higher difference score) for a male target than did the control females ($t(13)=3.17$, $p=0.003$). Thus, hormonal treatment (without concurrent CP55,940) successfully increased sexual motivation in this behavioral paradigm, similar to what we have previously reported (López et al., 2009, 2007). Within the oil-treated subjects, the 0 and 40 µg/kg dosage groups were not statistically different ($t(13)=0.62$, $p=0.27$). Similarly, there was no difference between drug groups for females treated with EB only ($t(14)=2.28$, $p=0.02$). However, among EB + P females, there was a significant difference between 0 and 40 µg/kg CP55,940 ($t(13)=4.46$, $p<0.001$). Specifically, the 0 µg/kg group had a higher mean difference score ($\overline{x}=41.1\pm12.2$) than the 40 µg/kg group ($\overline{x}=-25.5\pm9.1$). These significant group differences are marked on Fig. 2C.

Retreat difference score data were also analyzed using a 3 (target) × 3 (hormone) × 3 (drug) multi-factorial ANOVA ($\alpha=0.05$). No main effects or significant interactions were found. During the experimental phase, the subjects averaged 3.8 (±0.2) retreats for an empty goalbox, 3.5 (±0.2) retreats for a female target, and 3.7 (±0.2) retreats for a male target. Thus, subjects expressed a significant degree of locomotor activity within the runway (traversing its entire length, on average, more than 6 times per trial) regardless of hormone or drug condition.

4. Discussion

Three primary findings emerged from this experiment. First, hormonal treatment with both estradiol and progesterone increased sexual motivation for a male conspecific in sexually-naïve female rats. Second, pre-treatment with the potent cannabinoid agonist, CP55,940, dose-dependently attenuated sexual motivation in estrous females. Third, the higher dose of CP55,940 (40 µg/kg) also reduced social motivation for a conspecific female. These findings suggest that activation of CB_1 receptors interferes with the generation and/or expression of socio-sexual motivation in females that have been primed with ovarian hormones.

Although our female subjects were sexually-naïve prior to motivational testing and did not receive any sexual experience throughout the experiment, they nevertheless experienced a significant increase in sexual motivation after being primed with both estradiol and progesterone. We take this as further evidence that estrous female rats experience an unconditioned sexual attraction to adult male rats (Eliasson and Meyerson, 1975; Nofrey et al., 2008). More specifically, the generation of sexual motivation is not dependent upon prior incentive–reward associations that are formed during the copulatory act (Pfaus et al., 2001). We have previously reported that sexually-naïve male rats, as well, demonstrate unconditioned attraction to estrous females (López et al., 1999). Clearly, rats do not possess a *tabula rasa* with regards to their mate choice and sexual preference.

The increase in sexual motivation experienced by our female subjects following sequential administration of estradiol and progesterone was dose-dependently blocked by treatment with CP55,940. Sexual motivation in estrous females was slightly, but not significantly, reduced by a 20 µg/kg dose and completely attenuated by 40 µg/kg. This finding supports our hypothesis that cannabinoids have an inhibitory effect on behavioral estrus in female rats. We have previously demonstrated that the CB_1 receptor antagonist/inverse agonist, AM251, increases sexual motivation and proceptivity in estrous females, indicating that endocannabinoids may normally exert tonic inhibition over aspects of behavioral estrus (López et al., 2009).

Behavioral interpretations of cannabinoid effects are often complicated by two confounding variables: anxiety and locomotion. With regards to locomotion, it might be argued that the deficits in sexual motivation observed in the current experiment could be interpreted instead as deficits in motoric capacity. Treated females may have spent less time in the goalbox with the male target because they were less capable of traversing the runway. We do not feel that this is a convincing argument for two reasons. First, treatment with CP55,940 (at either dose) had no effect on proximity times for an empty goalbox. That the effects of the drug were limited to those conditions where the subject was presented with a social target supports a motivational interpretation. Second, CP55,940 had no effect on the subjects' retreats. Even subjects treated with the higher dose of CP55,940 continued to traverse the alley numerous times throughout the course of the trial. There was no observational evidence of significantly reduced locomotor function. Rather, the data indicate that treated subjects chose to spend less time in the goalbox, in close proximity to male and female targets, when compared to vehicle-treated controls.

As noted, CP55,940-treated subjects (40 μg/kg) not only expressed reduced sexual motivation for male conspecifics but also reduced social motivation for other females. This introduces the possibility that cannabinoid inhibition of sexual motivation, at least in the context of the current experiment, may be a reflection of a more general diminishment in social motivation. Over two decades ago, van Ree et al. (1984) noted that Δ^1-tetrahydrocannabinol dose-dependently reduced social interactions in dyadic encounters between male rats, and suggested that cannabinoids may selectively inhibit close, intimate contacts between conspecifics. Reduced motivation to engage in social interaction can be explained by two distinct theoretical mechanisms: conspecific cues may experience a reduction in positive incentive value, or acquire negative valence. Genn et al. (2004) have previously shown that 40 μg/kg CP55,940 significantly reduces the amount of time that male rats will spend physically engaged with one another, in a social interaction test that is particularly sensitive to manipulations of subject anxiety. It is possible that in the current experiment, 40 μg/kg CP55,940 indirectly reduced sexual motivation by eliciting a degree of social anxiety in the estrous female subjects. While hormone treatment enhanced the positive incentive value of male cues, drug treatment counteracted the behavioral manifestation of this by enhancing the negative incentive value of male cues.

It is interesting to note that drug treatment had no significant behavioral effect on female subjects who were not hormonally primed. Nonestrous females given 40 μg/kg CP55,940 did not show reduced proximity times for any of the goalbox targets. This finding could be taken as evidence that the drug, by itself, did not induce anxiety. Rather, the behavioral effects of CP55,940 only manifested themselves in females who were primed by estradiol and progesterone. There is accumulating evidence that cannabinoid effects are moderated by ovarian hormones. In a seminal paper, Rodríguez de Fonseca et al. (1994) noted that exogenous administration of estradiol and progesterone to OVX female rats induced significant upregulation of CB_1 receptors in the hypothalamus. This suggests the possibility that a hormonally-primed female brain may be more responsive to the effects of cannabinoid agents. Indeed, female mammals appear to be more sensitive to the behavioral effects of cannabinoids than males, and it is likely that this sexual dimorphism is linked to gonadal hormone activity (Craft, 2005; Craft and Leitl, 2008). Mani et al. (2001) have suggested that ovarian hormones adjust the sensitivity of certain neural processes to cannabinoids by modulating dopaminergic activity. Mani's group found that administration of THC to estradiol-primed females enhanced sexual receptivity, and that this effect was blocked by pre-treatment with either a progesterone or dopamine receptor antagonist. In contrast, we did not see any evidence that CP55,940 increased sexual motivation in our EB-only females. It is possible that sexual motivation and receptivity are differentially affected by cannabinoid treatment. However, our laboratory has now observed that both AM251, a cannabinoid antagonist, and CP55,940, a cannabinoid agonist, exert strong behavioral effects on estrous, but not nonestrous, females. Such findings do hint at possible progesterone-cannabinoid receptor cross-talk, as described by Mani et al. (2001).

We believe there is now significant evidence that cannabinoids affect the generation and/or expression of sexual motivation in female rats (Gorzalka and Hill, 2006). We have previously shown that a cannabinoid antagonist/inverse agonist increases sexual motivation in estrous females. The current experiment revealed that treatment with a potent CB_1 agonist significantly attenuated socio-sexual motivation in estrous females. It is possible that cannabinoid effects on social and sexual motivation are mediated, in part, by indirect effects on other motivational and emotional systems, such as anxiety. The precise mechanisms by which ovarian hormones and the endocannabinoid system interact in the regulation of behavior have only recently come under scientific scrutiny (e.g. Hill et al., 2007).

There is increasing evidence that the endogenous cannabinoid system helps modulate activity of the hypothalamic–pituitary–gonadal (HPG) axis (Murphy et al., 1998). For example, hypothalamic neuroendocrine cells synthesize and release endocannabinoids that inhibit GnRH secretion (Gammon et al., 2005). Intracerebroventricular administration of anandamide, an endocannabinoid, attenuates both GnRH and prolactin release in ovariectomized (OVX) female rats but increases plasma lutenizing hormone and prolactin in estradiol-primed females (Scorticati et al., 2004, 2003). Furthermore, in female rats, there are prominent estrous fluctuations in endocannabinoid content, CB_1 receptor gene expression, and CB_1 density within brain regions known to participate in the generation of estrous behavior (Bradshaw et al., 2006; González et al., 2000; Rodríguez de Fonseca et al., 1994). These findings collectively suggest that there is ongoing reciprocal regulation between the endocannabinoid system and female endocrine system that may be linked to the generation of reproductive behavior (López, in press).

If cannabinoid agents enter the prescription drug market in the near future, it is vital that we explore the many, disparate means by which pharmacological manipulation of the cannabinoid system could affect human psychology, behavior, and physiology. Our current research suggests that cannabinoid agonists might very well have a negative impact on women's libido, under certain hormonal and environmental circumstances. We are currently examining the long-term effects of chronic cannabinoid treatment, during both adolescence and adulthood, on the expression of behavioral estrus in female rats. Given the preponderance of marijuana use among teenagers and college students in the U.S., there is strong incentive to research cannabinoid effects on neuroendocrine system development and function using valid and reliable animal models (Rubino and Parolaro, 2008; Viveros et al., 2005).

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