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Cannabinoid receptor antagonism increases female sexual motivation

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ABSTRACT

The current experiments examined whether treatment with a CB1 antagonist/inverse agonist (AM251) affects sexual motivation, proceptivity, and receptivity in female rats. In experiment #1, 92 Long–Evans rats were tested for their socio-sexual motivation via a runway methodology. Motivation to approach and maintain close proximity to an empty goalbox, a female, and a male target was assessed following hormonal and drug treatment. Hormone treatments were: oil vehicle, 10 μg estradiol, and 10 μg estradiol+500 μg progesterone. Drug doses were 0, 2, and 4 mg/kg AM251 (IP, 60 min prior to testing). In experiment #2, 32 female subjects were tested for receptivity and proceptivity in a paced mating chamber. Subjects were given either a high (10 μg estradiol+500 μg progesterone) or low dose of hormones (2 μg estradiol+250 μg progesterone), and either vehicle or 2 mg/kg AM251. AM251 significantly increased sexual motivation for a male target in the runway in females primed with both estradiol and progesterone. AM251 also enhanced lordosis (in low hormone females) and increased hop-darts. These findings suggest that endocannabinoids tonically inhibit estrous behaviors. Cannabinoid antagonists could serve as new treatment option for women suffering from abnormally low libido.

1. Introduction

The discovery of endogenous cannabinoids and a surprisingly high density of cannabinoid receptors in the mammalian brain has led to a flurry of research over the past decade into the functional properties of this system (Vettor et al., 2008). Endocannabinoids help regulate a number of motivated behaviors and emotional states, including feeding, pain, anxiety, and drug-seeking (Chaperon and Thiebot, 1999; Gardner, 2005; Pagotto et al., 2006). Several cannabinoid agonists (e.g. dronabinol, nabilone) and antagonists (e.g. rimonabant) have been promoted for potential therapeutic use in human patient populations. Recent work has suggested that the endocannabinoid system also plays an important role in reproductive behavior in both males and females, although the specific nature of this involvement remains unclear. Scientific understanding of this issue will increase in importance as cannabinoid agents increasingly enter the clinical arena and prescription drug market.

In male rats, cannabinoid agonists tend to inhibit both copulatory performance and sexual motivation. Acute treatment with Δ^9-THC reduced male sexual incentive motivation in an approach behavior test (Navarro et al., 1993), while the endocannabinoid, anandamide (AEA), reduced intromission frequency and increased ejaculation latencies (Martinez-Gonzalez et al., 2004). Endocannabinoids also exert inhibitory control over penile erection and male sexual arousal. Administration of cannabinoid antagonists, like SR141716A and AM251, 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; Melis et al., 2006; Succu et al., 2006).

Research on cannabinoid modulation of female sexual behavior has been more conflicting. Prior to the discovery of the endocannabinoid system, Gordon et al. (1978) demonstrated that a low dose of Δ^9-THC enhanced lordosis in estradiol-primed female rats, while a high dose interfered with expression of receptivity. Such dose-dependent patterns are common in the cannabinoid literature, due to the anxiogenic, sedating, and motor-inhibitory effects of high doses. Turley and Floody (1981) noted that Δ^9-THC stimulated both receptivity (lordosis) and proceptivity (ultrasonic vocalizations) in female hamsters primed with estradiol. These early studies suggested that cannabinoid agonists could serve as progesterone "surrogates," a premise more recently explored by Mani et al. (2001). However, contradictory to this hypothesis, Ferrari et al. (2000) found that the potent cannabinoid agonist, HU210, dose-dependently attenuated both lordosis and proceptive displays in estrous female rats.

Work by Mani et al. (2001) has reinvigorated the debate over possible cannabinoid mediation of behavioral estrus. The authors demonstrated that intracerebroventricular administration of Δ^9-THC increased lordosis quotients in females treated solely with estradiol, to levels equivalent of females treated with both estradiol and

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progesterone. This enhancement of receptivity was blocked by concurrent administration of a CB1 antagonist, a progesterone receptor (PR) antagonist, or a dopaminergic D1 antagonist. Furthermore, treatment of estradiol/progesterone primed females with the CB1 antagonist, SR141716A, significantly attenuated lordosis quotients. This research suggested that cannabinoid modulation of estrous behavior was dependent upon CB1-PR-D1 receptor cross-talk. No measures of proceptivity or sexual motivation were recorded in these experiments.

Cannabinoid effects on estrous behavior are further complicated by cyclic fluctuations in both endocannabinoid activity (Bradshaw et al., 2006; Gonzalez et al., 2000) and CB1 receptor densities (Rodriguez de Fonseca et al., 1994). For example, production and release of the endocannabinoids, 2-AG and AEA, peak in the female hypothalamus during diestrus, but diminish as the female enters into behavioral estrus. The endocannabinoid system may form part of an intricate feedback system that helps regulate the appearance and duration of estrous behaviors, as well as modulates the experience of sexual reward during copulation.

The current research was conducted to specifically examine the role that endocannabinoids may play in regulating female sexual motivation. By and large, most research on the neurochemical basis of female sexual behavior has focused upon receptivity and the lordosis reflex, primarily due to the ease of measuring this behavior. However, in recent years it has become increasingly clear that receptivity does not adequately model women's sexuality. There has been a significant push to develop animal models of human sexual desire, as a means of assessing the pro- or anti-sexual effects of various pharmacological compounds (Agmo and Ellingsen, 2003; Agmo et al., 2004; Pfaus et al., 2003). Our laboratory uses a runway methodology that allows for the assessment of incentive-motivation in male or female rats, does not require reinforcement training, and does not present subjects with more than one stimulus target at the same time (unlike preference methodologies). Using this model, we have previously explored both unconditioned and conditioned sexual motivation in male rats (Lopez and Ettenberg, 2001, 2000, 2002; Lopez et al., 1999), and the effects of hormone and drug treatment on sexual motivation in female rats (Lopez et al., 2007).

In two experiments, we tested the effect of the cannabinoid antagonist/inverse agonist, AM251, on female estrous behaviors. AM251 has been used in recent years to examine the role of endocannabinoids in feeding (Chambers et al., 2004), anxiety (Hill et al., 2007), and drug-seeking behavior (Shoaib, 2008). Precisely how AM251 and its structural relative, SR141617A, affect cannabinoid activity *in vivo* to modulate behavior remains to be determined. Recent *in vitro* studies have suggested that at lower doses, AM251 may serve as a simple "neutral" antagonist, with inverse agonist activity emerging at higher doses (reviewed in Pertwee, 2005). The current study adopted two doses of AM251 (2 mg/kg and 4 mg/kg) that suppress ingestive behavior in free-feeding rats, which may be indicative of inverse agonist activity (McLaughlin et al., 2003).

Sexual incentive motivation was assessed in the runway using a male target. Subjects also ran for an empty goalbox, which controlled for exploratory motivation and potential locomotor effects, and a female target, which controlled for social motivation. In a follow-up experiment, proceptivity and receptivity were assessed using a paced mating procedure, in which female subjects were allowed to control the frequency of mounts and intromissions (Erskine, 1989; Paredes and Vazquez, 1999). Paced mating provides a more naturalistic and valid assessment of female sexual behavior under controlled laboratory conditions. If endocannabinoids support behavioral estrus, as the work of Mani et al. (2001) suggests, then we would expect AM251 to attenuate sexual motivation, lordosis, and proceptive displays in hormonally primed females. If endocannabinoids normally exert tonic inhibition, then AM251 should enhance these estrous behaviors.

2. Method

2.1. Subjects

All animals were obtained from Charles River Laboratories (Wilmington, MA). Females were ovariectomized (OVX) at Charles River one week prior to arrival in our laboratory. All females were given at least one additional week to recover from surgery and adjust to the vivarium environment.

Subjects for experiment #1 were 92 OVX female Long–Evans rats, approximately 75 days old at the start of testing. Eleven sexually experienced, male Long–Evans rats (75–110 days old) served as sexual partners during copulatory tests. Two different sexually experienced, male Long Evans rats (75–110 days old) served as goalbox targets to induce sexual motivation in subjects. Two OVX female Long–Evans rats (75–110 days old) served as goalbox targets to induce social motivation.

Subjects for experiment #2 were 32 OVX female Long–Evans rats, approximately 120 days old at the start of testing. Eight male Long–Evans rats (120 days old) served as mating partners. These males had previously been screened for copulatory potency (i.e. they mounted within 1 min and achieved an ejaculation within 10 min of being paired with an estrous female).

Males were individually housed in plastic tubs within a secure, temperature-controlled (23±2 °C) vivarium. Females were housed in pairs within the same vivarium but not in close proximity to the males. Food and water were provided *ad libitum*. Animals were maintained under a reverse 12:12 light–dark schedule (lights on 22:00–10:00 h). All animals were handled daily by experimenters for one week prior to any behavioral testing. The care and use of animals, and all aspects of the experimental protocol, were reviewed and approved by the campus IACUC (Institutional Animal Care and Use Committee) for compliance with the National Institute's 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 a propylene glycol vehicle. Both hormones were injected subcutaneously at a volume of 0.1 ml. AM251 (Tocris Biosciences, Ellisville, MO) was prepared in a vehicle of 10% DMSO, 10% Tween-80, and 80% physiological saline (as in McLaughlin et al., 2005). Subjects in both experiments received either vehicle or AM251 intraperitoneally (IP) 60 min prior to behavioral testing. All injections were administered in a volume of 1 ml/kg. Experiment #1 used both a 2 and 4 mg/kg dose, while experiment #2 used only a 2 mg/kg dose. These doses have been shown to significantly affect other motivated behaviors in the rat, such as feeding, without significantly suppressing or enhancing locomotor function (McLaughlin et al., 2003). Recent evidence indicates that AM251 does not significantly affect locomotor activity, even at high intracranial doses (de Oliveira Alvares et al., 2005).

2.3. Runways

Motivational testing took place within two identical straight-arm runways consisting of a startbox (25×25×20 cm), an alley (160×10×20 cm), and a cylindrical Plexiglas goalbox (50 cm diameter×30 cm height). Fig. 1 depicts a line-drawing of the runway apparatus. Removable Plexiglas doors were located between the startbox and alley and between the alley and goalbox. Within the goalbox, a removable Plexiglas partition divided the arena into two semicircular halves. Thirty-five 1-cm diameter holes drilled into the partition allowed air to pass between the two sides of the goalbox. This partition prevented tactile contact between subject and target

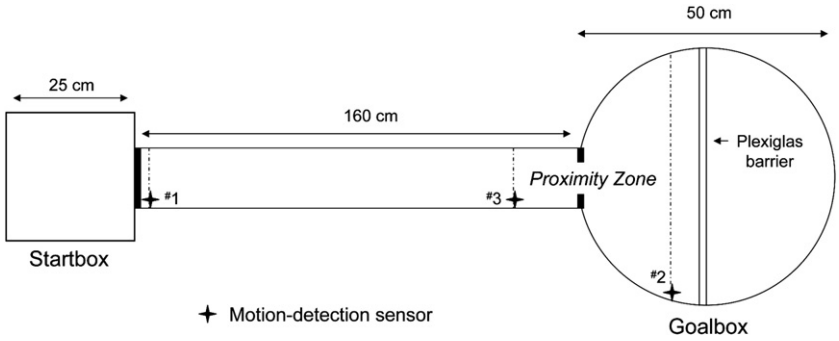

Fig. 1. The runway apparatus used to assess socio-sexual motivation in experiment #1. Sensors #1 and #2 allowed for the measurement of run time, while sensors #2 and #3 allowed for the measurement of proximity time.

during motivational testing, although visual, auditory, and olfactory cues were accessible.

Three infrared photocell emitter-detector sensor pairs were placed within the apparatus to detect subject motion. Sensor #1 was located just outside the startbox and was triggered when the subject entered the alley. Sensor #2 was located within the goalbox (15 cm from the entry) and was triggered when the subject's entire body was within the goalbox. These two sensor pairs were linked to an electronic timer that recorded "run time." This timer started when the subject triggered the sensor #1 and stopped when the animal triggered sensor #2. Sensor #3, located within the alley (25 cm from the goalbox entry), became responsive only after an initial goalbox entry. Sensor #2 and #3 allowed for measurement of subject "proximity time." An electronic timer started when the subject first entered the goalbox and triggered sensor #2. If the subject's entire body left the goalbox and triggered sensor #3, the timer stopped. If the subject re-entered the goalbox and triggered sensor #2, the timer would start again. This continued for a period of 3 min, following the initial entry of the subject into the goalbox.

This apparatus has been previously utilized to assess socio-sexual motivation in both male (Lopez and Ettenberg, 2001, 2000, 2002; Lopez et al., 1999) and female rats (Lopez et al., 2007; Nofrey et al., 2008).

2.4. Paced mating chambers

Two paced mating chambers were used for copulatory tests in experiment #2. Each measured 60×40×40 cm, and was constructed from wood and Plexiglas. A removable wooden barrier divided the chamber in half, but allowed passage between the sides through three equally-spaced 4×4 cm holes. These holes were large enough to let a full-grown female rat through, but not an adult male. A clear Plexiglas roof and front allowed for behavioral observation and video recording. Prior to use, the floor was covered with a layer of corn-cob bedding which was replaced for each copulatory session.

2.5. Procedure

2.5.1. Experiment #1: effects of AM251 on sexual motivation

All 92 female subjects were given a single sexual experience with an adult male partner, several days before motivational testing in the runway. Four cylindrical sex arenas (53 cm diameter, 60 cm height) were used for this purpose. All testing occurred under red-light conditions, during the dark portion of the animals' photoperiods. Females were given 10 μg EB (48 h prior) and 500 μg P (5 h prior) to induce behavioral estrus. They were then paired with a male in the sex arena until the male ejaculated or 30 min passed. If the male did not mount within 10 min, another male was substituted and testing recommenced. All females were mounted numerous times and all but two received an ejaculation under these test conditions. Experimenters recorded the time to ejaculation (ejaculation latency), as well as the number of mounts the female received during the test session. The mean (±SEM) ejaculation latency during sexual tests was 495 (±43) seconds, and the mean number of mounts was 20 (±1). After the male had ejaculated, both male and female were returned to the vivarium.

Subjects were given two habituation sessions (10 min each) within an empty runway on consecutive days. Baseline testing then began. All runway testing took place under red-light illumination during the 2nd third of the dark phase of the animals' photoperiod. Over the next six days, subjects were tested for their motivation to approach and maintain close proximity to one of the three different goalbox targets: an empty goalbox, an OVX (nonestrous) female, or an adult male. Subjects were tested in a nonestrous state throughout the baseline phase. On any given day, all subjects ran for the same target in the goalbox; only one trial per day per subject was conducted. Thus, subjects ran for each goalbox target twice during the baseline phase. The order of goalbox targets was randomly determined.

Prior to a day's trials, the assigned target (if a female or male conspecific) was confined within the goalbox for a period of 10 min. A Plexiglas partition was then introduced into the goalbox with the target placed on the side farthest from the goalbox entrance. A female subject was placed into the goalbox on the opposite side of the partition from the target, and given 2 min to investigate. The subject was then swiftly removed from the goalbox and immediately placed within the startbox. The two removable doors were lifted, and the subject was allowed to traverse the alley and re-enter the goalbox. "Run time" was defined as the amount of time (in seconds) it took the subject to enter the goalbox after leaving the startbox. Presumably, a lower run time indicates greater incentive-motivation. "Proximity time" reflects the subject's desire to stay in close physical proximity to the goalbox target and was defined as the amount of time the subject spent in the goalbox (following her initial entry), out of a possible 3 min. A higher proximity time indicates greater incentive-motivation. After this three-minute period expired, the subject was removed from the runway and returned to her homecage. The runway was quickly wiped down to remove any urine or feces left by the subject prior to initiating the next trial. This procedure was repeated until all animals were tested. The order of subjects run was kept constant throughout the experiment. The entire runway apparatus was cleaned with a 20% ethanol solution at the end of each day's trials.

Following completion of the baseline phase, subjects were divided into six experimental groups (n=12–14/group) such that mean baseline run times and proximity times were approximately equal between groups. Subjects were then re-tested in the runway for their motivation to approach the same three goalbox targets (empty, female, male) under one of three hormonal conditions (oil, EB, or EB+P) and one of two drug conditions (0 or 2 mg/kg AM251). EB injections (10 μg/subject) were administered 48 h prior to behavioral testing. P injections (500 μg/subject) were administered 5 h prior to testing. Drug injections (IP) were given 1 h prior to behavioral testing.

Preliminary analysis of our data revealed that AM251 only affected sexual motivation in fully estrous (EB+P) females. We therefore decided to test an additional group of EB+P subjects with a 4 mg/kg dose of AM251. While an optimal design calls for pairing the 4 mg/kg dose with the vehicle and EB hormone conditions as well, the prohibitive cost of the drug motivated us to concentrate our efforts on the most interesting and relevant potential interaction.

Subjects ran a single experimental trial for each goalbox target. These trials occurred, by necessity, fours days apart due to the induction of behavioral estrus in several subject groups. Such a treatment regimen requires that at least three days separate test periods, mimicking the natural estrous cycle of the female rat. On the second day after each experimental trial, 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).

2.5.2. Experiment #2: effects of AM251 on receptivity and proceptivity

Results from experiment #1 indicated that AM251 increased sexual motivation in EB+P primed females. As a follow-up experiment, we assessed the effect of AM251 on receptivity (lordosis) and proceptivity (hop-darts) in ovariectomized females given both estradiol and progesterone. For this experiment, an estradiol-only condition was not included, since AM251 had no effect on estradiol-only females in experiment #1.

Female subjects received a 15-minute habituation session in the paced mating apparatus several days prior to testing, and all females demonstrated the ability to pass through the barrier. Male partners had previously been trained to engage in sexual behavior within the arenas. Behavioral tests lasted 15 min. Females were placed in the paced mating chamber first and allowed to habituate for 5 min before a male partner was introduced on the opposite side of the barrier. Eight sexually-experienced, adult Long–Evans males were used as copulatory partners. These same 8 partners were paired with each of the experimental groups, controlling for potential differences in male responsiveness. Sex tests occurred every other day, such that each male had 48-hours of rest between sessions.

Subjects were divided into 4 experimental groups, based on a 2 (hormone dose)×2 (drug dose) design. Two groups received high, supraphysiological hormone doses of 10 μg EB+500 μg P prior to testing (as in experiment #1). The two other groups received lower, physiological doses of 2 μg EB+250 μg P. This lower dosage regimen was chosen since receptivity and proceptivity may maximize following high hormone treatment, leaving little room for potential drug enhancement (Paredes and Agmo, 2004; Tennent et al., 1980). One group in each hormone condition received 2 mg/kg AM251 (IP) 1 h prior to testing, while the other two groups received vehicle.

A single experimenter, blind to the experimental condition of the female, recorded the following dependent variables: 1) lordosis rating (LR), 2) lordosis quotient (LQ), 3) proceptivity, and 4) number of pacing exits. Lordosis rating was assessed as in Hardy and DeBold (1971), using a 4-point scale to score every female response to a male mount attempt (0=no lordosis, 1=marginal, 2=normal, 3=exaggerated). Lordosis quotient was defined as the percentage of male mounts that the female responded to with any degree of lordosis (1–3, on the aforementioned scale). Both LR and LQ were based on a variable number of mounts for each subject, due to our desire to keep session length consistent. The minimum required was 7 and the maximum was 20. Four subjects were dropped from subsequent analysis because they did not receive at least 7 mounts during sexual testing. The mean (±SEM) number of mounts used to calculate LR and LQ in the remaining subjects was 16 (±0.9). Proceptivity was assessed by counting the number of individual hop-darts that the female engaged in throughout the copulatory session. Hop-darts were behaviorally defined as in Tennent et al. (1980). A pacing exit was counted each time the female subject left the male side of the chamber. This variable was used as a general behavioral index of the copulatory session, and one that could possibly signal whether the drug was having an adverse effect on the subject's sexual experience. We did not predict that AM251 would significantly affect pacing exits in this experiment. While this variable is not a prototypical paced mating variable (such as return latency), the focus of this experiment was not to assess aspects of paced mating, *per se*, but rather receptivity and proceptivity within the context of a paced mating session.

Prior to each copulatory test, the paced mating chamber was cleaned with a 20% ethanol solution and fresh corn-cob bedding was applied.

3. Results

3.1. Experiment #1

Two subjects were excluded from statistical analysis because their experimental RT and PT scores were more than three standard deviations away from the overall mean. This experiment utilized a 3 (goalbox target)×3 (hormone condition)×2 (drug dose) design, with hormone and drug serving as between-subject variables. A mixed-factorial analysis of variance (ANOVA) was conducted on both the run time and proximity time data (using an alpha of 0.05). Group averages are expressed as mean±SEM.

Analysis of subject run time yielded a significant interaction between goalbox target and drug ($F(2,142)=4.4$, $p=.01$). We then conducted a 2 (drug)×3 (hormone) ANOVA on the RT data for each goalbox target. There were no main effects or interactions for the empty goalbox and female target. There was a marginally significant effect of drug on the male target data ($F(1,71)=3.7$, $p=.057$), with drug-treated animals running slower (22.4±4.4 s) than vehicle-treated animals (13.5±2.1 s). Notably, there was no significant effect of hormone on RT for a male target, suggesting that RT was not serving as an index of subject motivation in this experiment. Fig. 2 displays subject run times for a male target. Non-estrous (oil) control females did not significantly differ from estrous (EB+P) control females. Additionally, there were no significant differences between vehicle-treated subjects and AM251-treated subjects within each of the three hormonal conditions (independent two-sample *t*-tests, $p=.05$).

With regards to proximity time (PT), there was a significant main effect of target ($F(2,142)=35.06$, $p<.001$), a target×hormone interaction ($F(4,142)=4.14$, $p=.003$), and a target×drug interaction ($F(2,142)=3.74$, $p=.03$). To further examine the nature of these interactions, we

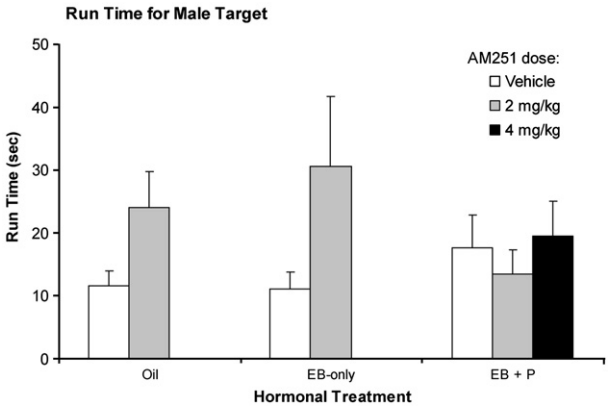

Fig. 2. The effect of hormone and drug treatment on run time for a male target in 7 groups of subjects ($n=12$–14/group). Hormone treatments consisted of vehicle (oil), 10 μg estradiol (EB-only), or 10 μg estradiol+500 μg progesterone (EB+P). Drug treatments were vehicle, 2, or 4 mg/kg AM251. Differences in run time did not appear to reflect changes in motivation.

examined PTs for each goalbox target separately. A 2 (drug)×3 (hormone) ANOVA was conducted on the PT data for each goalbox target. There was no effect of hormonal condition or drug treatment on motivation for an empty goalbox. Similarly, for the female target, there was no effect of hormone or drug. However, for a male target there was a significant main effect of hormone ($F(2,71)=6.41$, $p=.003$), and post-hoc analysis using Tukey's HSD revealed that subjects treated with both EB and P expressed higher PTs compared to both oil-treated subjects ($p=.003$) and EB-only subjects ($p=.04$). There was also a significant main effect of drug ($F(1, 71)=8.37$, $p=.005$), with AM251-treated subjects expressing higher PTs compared to vehicle-treated subjects.

Fig. 3A–C shows subject proximity times for the empty goalbox (A), female target (B), and male target (C), for all seven experimental groups (including the 4 mg/kg AM251 dosage group). To specifically test our *a priori* hypothesis that AM251 would affect sexual motivation, we conducted a small set of planned comparisons within the male target data set (Fig. 3C). Within each of the three hormonal conditions, vehicle-treated subjects were compared to AM251-treated subjects using an independent two-sample *t*-test (two-tailed). We also compared the non-estrous (oil) control group to the estrous (EB+P) control group to confirm that our hormonal manipulation successfully increased sexual motivation (one-tailed because of our directional hypothesis).

Control females primed with EB+P did express significantly higher PTs compared to females given just oil ($t(25)=2.3$, $p=.01$). Thus, hormonal treatment (without concurrent AM251) successfully increased sexual motivation in this behavioral paradigm, similar to what we have previously reported (Lopez et al., 2007). AM251 treatment did not affect PT in oil-treated females ($t(23)=1.8$, $p=.08$) or EB-only females ($t(23)=0.4$, $p=.66$). However, both doses of AM251 significant increased PT in females primed with EB+P: 2 mg/kg ($t(25)=2.9$, $p=.008$) and 4 mg/kg ($t(25)=2.5$, $p=.02$). These data suggest that treatment with AM251 increased sexual motivation only in fully estrous females.

3.2. Experiment #2

As noted earlier, 4 subjects were excluded from statistical analysis because they did not receive a minimum of 7 mounts during testing. There were no significant effects of hormone or drug on female exits. Across all four groups, females exited from the male side a mean of 8.9 (±1.1) times per test session.

Fig. 4A–B displays the effects of hormone and drug treatment on receptivity, as expressed by both lordosis quotient (A) and lordosis rating (B). A 2 (hormonal condition)×2 (drug dose) ANOVA was conducted on these data. For lordosis quotient (LQ), there was a significant main effect of hormone ($F(1,24)=5.39$, $p=.03$), as well as drug ($F(1,24)=4.41$, $p=.05$), and a significant drug×hormone interaction ($F(1,24)=5.1$, $p=.03$). Post-hoc analysis using Tukey's HSD ($\alpha=.05$) revealed that low hormone females without AM251 treatment possessed significantly lower LQ scores than each of the other three groups. There was a significant improvement in LQ following AM251 treatment, but only in females primed with the lower hormone dose. Analysis of lordosis rating (LR) revealed no main effects, but a marginally significant hormone×drug interaction ($F(1,24)=4.05$, $p=.056$). Tukey's HSD did not reveal any significant differences between individual groups, but the overall pattern of results suggests that (similar to LQ) AM251 enhanced LR in females treated with the lower hormonal dose.

Fig. 5 displays the effects of hormone and drug treatment on proceptivity, as expressed by the total number of hop-darts emitted by the female subject during the 15-minute copulatory test. A 2×2 ANOVA revealed a significant main effect of drug on hop-darts ($F(1,24)=4.10$, $p=.05$) but no interaction. Across both hormonal conditions, subjects made significantly more hop-darts while under the influence of AM251 (26.4±3.3) than after vehicle administration (17.3±3.1).

4. Discussion

Systemic administration of the CB1 antagonist/inverse agonist, AM251, significantly increased sexual motivation in estrous female rats as assessed by proximity time in a runway procedure and proceptive displays in a paced mating test. Experiment #1 demonstrated that females primed with estradiol (EB) and progesterone (P) and then treated with AM251 prior to testing spent a significantly greater amount of time around a male target compared to undrugged, estrous females (see Fig. 3C). In contrast, social motivation (for a female target) and exploratory motivation (for an empty goalbox) were not affected by either hormonal or drug treatment (see Fig. 3A

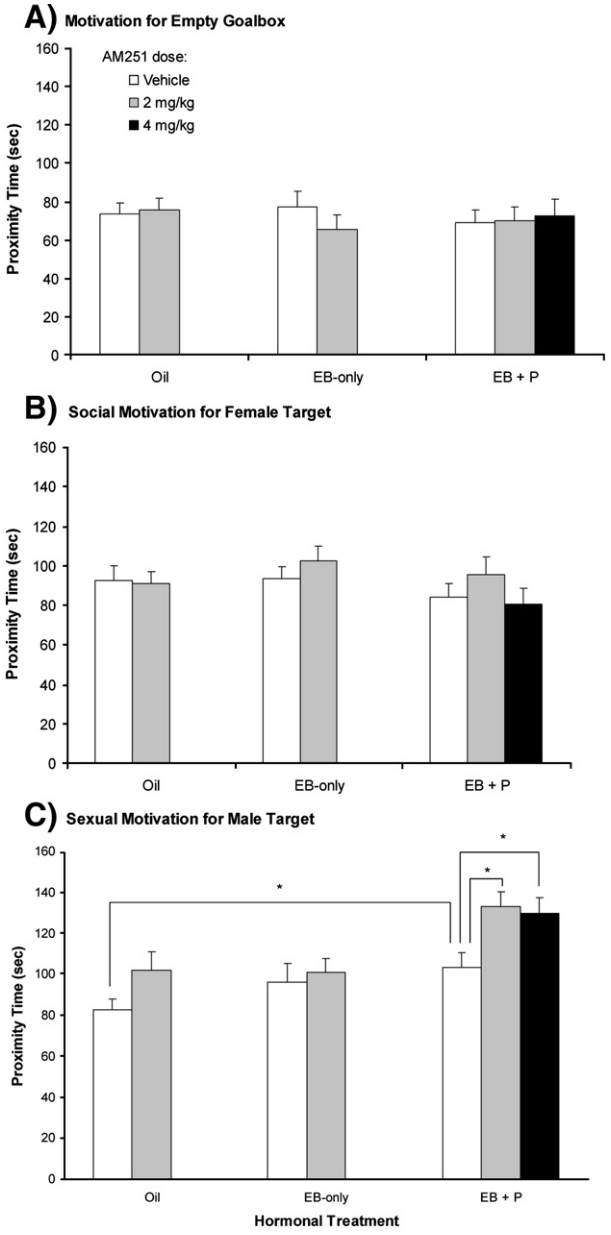

Fig. 3. (A–C). The effect of hormone and drug treatment on motivation to maintain proximity with A) an empty goalbox, B) a female target, and C) a male target in 7 groups of subjects ($n=12$–14/group). Hormone treatments consisted of vehicle (oil), 10 μg estradiol (EB-only), or 10 μg estradiol+500 μg progesterone (EB+P). Drug treatments were vehicle, 2, or 4 mg/kg AM251. * indicates a significant difference (independent two-sample *t*-test, $p\leq.05$).

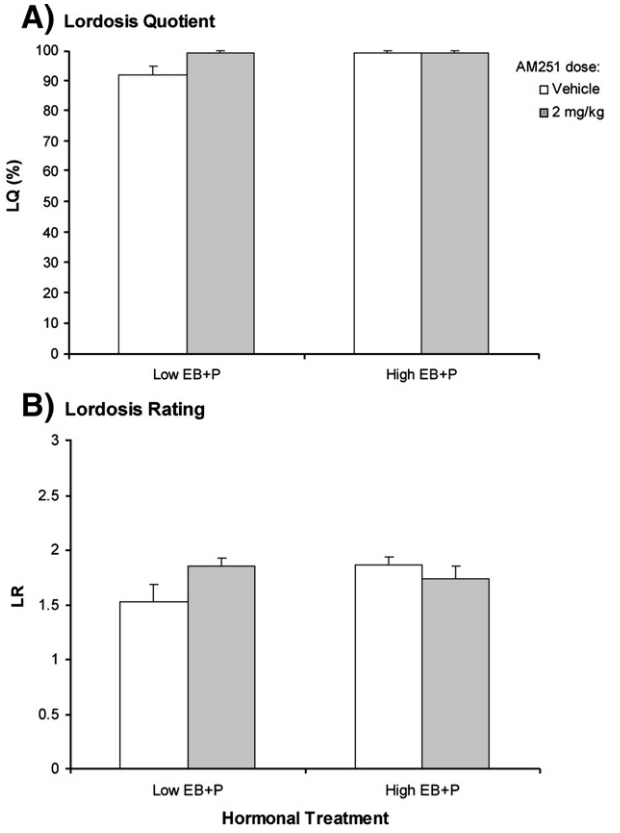

Fig. 4. (A and B). The effect of hormone and drug treatment on lordosis quotient (A) and lordosis rating (B). Receptivity was assessed in a 15-minute paced mating test. Subjects (n=8/group) received either a low/physiological dose of hormones (2 μg EB+250 μg P) or a high/supraphysiological dose (10 μg EB+500 μg P). Drug treatment was either vehicle or 2 mg/kg AM251. For both LQ and LR, there was a significant hormone×drug interaction, such that AM251 enhanced lordosis in females that received the lower hormonal dose, but had a negligible effect on females treated with the higher dose.

and B). These findings suggest that blocking CB1 receptors releases sexual motivation from inhibitory control by endocannabinoids, but only when a female has entered into behavioral estrus. AM251 did not increase sexual motivation in females treated solely with estradiol; endocannabinoid mediation of sexual motivational processes seems linked to progesterone activity. In our second experiment, AM251 treatment caused hormonally-primed females to engage in a significantly greater number of proceptive displays (see Fig. 5). Many researchers, based upon Beach's (1976) formulation, view proceptivity as a manifestation of sexual motivation. If this is true, than both experiment #1 and experiment #2 provide independent evidence for cannabinoid modulation of female sexual desire.

While proximity time served as a reliable measure of socio-sexual motivation in the current study, run time (RT) did not. We have previously used RT to assess sexual motivation both in male rats (Lopez et al., 1999) and female rats (Nofrey et al., 2008). However, as can be seen in Fig. 2, we were not able to demonstrate an estrous effect in experiment #1. Non-estrous control females ran unexpectedly fast for a male target (11.6±2.3 s) and hormonal treatment (EB+P) led to a slight slowing of run time (17.6±5.2 s). Because of this failure to show a decrease in RT for a male target following hormonal treatment, RT could not be used as a valid measure of subject motivation in the current experiment. Run time, in general, demonstrates greater within-group variance when compared to proximity time (Lopez et al., 2007), possibly because it is more affected by uncontrolled events or stimuli (such as loud noises) that happen at the start of a trial. Proximity time tends to be more stable, since subjects have 3 min to express their motivational state. The value of proximity time as a motivational variable has been well-established by Agmo et al. (2004), who have also argued that proximity measures are less likely to be influenced by non-motivational factors, such as motoric disruption.

Experiment #2 also demonstrated that AM251 enhanced lordosis in females primed with lower, physiological doses of estradiol and progesterone (see Fig. 4). Drug treatment had no effect on females that received the higher hormonal doses, presumably because of a ceiling effect where maximal receptivity was achieved via supraphysiological hormone treatment. Females treated with 2 μg EB+250 μg P and AM251 (2 mg/kg) exhibited levels of receptivity equivalent to subjects treated with 10 μg EB+500 μg P. These results are in contrast to those of Mani et al. (2001) who found that treatment of hormonally-primed females with the cannabinoid antagonist, SR141716A, significantly attenuated receptivity (LQs dropped from 85% to 30%). The reason for this discrepancy is unclear, but there are a number of methodological differences between their study and our own. In their experiment, females were given 2 μg EB (SC) and 2 μg P (ICV, 30 min prior to testing), whereas in the current study, both EB and P were administered systemically. Their cannabinoid antagonist was also administered centrally, limiting its effect to brain CB1 receptors – although it seems unlikely that the results of our experiments could be explained by peripheral cannabinoid activity. Finally, Mani et al. assessed receptivity in a non-paced mating procedure, and ended their sex tests after the male mounted the female ten times. The current experiment used a paced mating paradigm of set-length (15 min) which allowed for a greater degree of female control over the frequency of intromissions. It is possible that cannabinoid antagonists/inverse agonists facilitate receptivity only if the female is allowed to determine when mounts occur. Future research will hopefully address the potential interaction between pacing and cannabinoid modulation of estrous behavior.

Together, these findings suggest that endocannabinoids normally play an inhibitory role in the regulation of estrous behaviors, including approach-solicitation behavior, proceptivity, and lordosis. Levels of both endocannabinoids and CB1 receptors within the female hypothalamus drop significantly between diestrus and late proestrus, when sexual behavior is initiated (Bradshaw et al., 2006; Gonzalez et al., 2000; Rodriguez de Fonseca et al., 1994). Given the central importance of the hypothalamus in regulating mammalian sexuality, it is possible that this cyclic reduction in cannabinoid activity helps "release" estrous behavior around the time of follicular maturity.

Interestingly, Rodriguez de Fonseca et al. (1994) found that exogenous administration of acute estradiol and progesterone to ovariectomized females led to a significant increase in hypothalamic CB1 receptors – a level higher than any seen during a "natural" estrous

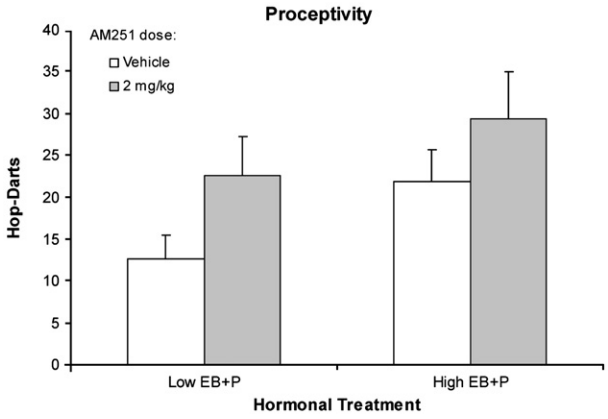

Fig. 5. The effect of hormone and drug treatment on proceptivity, as assessed by the number of hop-darts emitted during a 15-minute paced mating test. Subjects (n=8/group) received either a low/physiological dose of hormones (2 μg EB+250 μg P) or a high/supraphysiological dose (10 μg EB+500 μg P). Drug treatment was either vehicle or 2 mg/kg AM251. Across both hormone conditions, AM251 significantly increased the number of hop-darts displayed by females ($F(1,24)=4.10$, $p=.05$).

cycle. This enhancement was progesterone dependent, suggesting that PR-activity elicits CB1 upregulation. This may help explain why, in the current study, AM251 only affected females treated with both estradiol and progesterone. In ovariectomized females, estradiol and progesterone treatment may induce estrous behaviors (somewhat counter-intuitively) in a context of increased cannabinoid receptor density and endocannabinoid activity. It is possible that endocannabinoids constitute part of a negative feedback system that controls the occurrence and duration of female estrous behavior.

While endocannabinoids may directly modulate estrous behavior via a hypothalamic mechanism, it is also possible that they influence copulatory responses through their effects on other hormone and neurotransmitter systems. Cannabinoid agonists significantly blunt the release of numerous hypothalamic and pituitary hormones that may play a role in mediating sexual behaviors (for a review, see Murphy et al., 1998). Δ^9-THC inhibits GnRH release from the hypothalamus, indirectly attenuating LH and FSH release from the anterior pituitary. Δ^9-THC and other cannabinoid agonists also have an inhibitory effect on prolactin release from the posterior pituitary (e.g. Hughes et al., 1981). There is evidence that both GnRH and prolactin facilitate estrous behaviors, especially lordosis, in female rats (Drago and Lissandrello, 2000; Sakuma and Pfaff, 1980). Blockade of CB1 receptors by AM251 could elicit an increase in the release of these hormones, which subsequently enhance estrous behaviors. On a similar note, administration of a cannabinoid antagonist increases the firing rate of oxytocin neurons in the supraoptic nucleus of the hypothalamus (Leng et al., 2005; Sabatier and Leng, 2006). There is evidence showing that oxytocin plays an important role in both initiating and maintaining female estrous behaviors, including proceptivity (Caldwell et al., 1986; Pedersen and Boccia, 2002).

Finally, endocannabinoids may mediate sexual behavior by influencing endogenous opioids. There is significant cross-talk between these two systems (Corchero et al., 2004; Robledo et al., 2008; Vigano et al., 2005) that plays a functional role in motivated behaviors, such as feeding and drug-seeking (Cota et al., 2006; Fattore et al., 2004). Antagonism of central cannabinoid receptors may reduce topic opioid activity, which normally inhibits female sexual behavior (Pfaus and Gorzalka, 1987). However, opioid modulation of female sexual behavior varies depending upon receptor subtype and neural locus (see, for example, Acosta-Martinez and Etgen, 2002), making it difficult to predict how a cannabinoid antagonist might affect opioid modulation of estrous behavior.

Research on the function of endocannabinoids is still in its infancy. However, the pervasiveness of this modulatory system speaks to its biological importance, and perhaps hints at the increased role that legal, prescribed cannabinoid agents will play in our society. The current research suggests that cannabinoid antagonists/inverse agonists, like SR141617A (Acomplia), which are currently being assessed as anti-obesity agents, may also modestly stimulate human libido. Up to one-tenth of adult men and one-third of adult women in the US suffer from inhibited sexual desire associated with marked distress or interpersonal difficulty (Basson, 2006; Laumann et al., 1999; Warnock, 2002) and effective pharmacotherapy is currently non-existent. Cannabinoid antagonists could be a novel therapeutic option for women suffering from low libido due to the hormonal alterations that occur following menopause, the symptoms of a psychiatric illness, or the negative side-effect of pharmaceutical treatment.

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