Ovarian Hormone Modulation of Supraspinal THC-Induced Analgesia

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As thesis advisor for Alisha McBride, I have read this paper and find it satisfactory:

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Peggy,

I approve.

Thanks,

Rebecca

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Précis

One third of Americans suffer from a painful condition that negatively affects their quality of life. Considering this statistic, it is imperative that we investigate mechanisms of pain and develop more effective drugs for pain management. In recent years, attention has been drawn to marijuana for pain management. Numerous studies have shown that compounds derived from marijuana, also known as cannabinoids, reduce pain in humans. The main psychoactive component in marijuana is THC.

Women are more likely than men to be afflicted with a painful condition during their lives, even when excluding pain caused from menstruation, pregnancy and labor. For example, women have a higher incidence of fibromyalgia, osteoarthritis, irritable bowel syndrome, and multiple sclerosis. Unfortunately, preclinical studies of pain and pain management rarely take this sex difference into account: in 8 out of 10 biomedical fields, research studies are more likely to use solely male subjects or a combination of males and females, as opposed to focusing on females. This is a detrimental practice in preclinical research because male and female physiology differ and do not necessarily respond to drugs the same way. For example, female rats are more sensitive than males to the pain-relieving effects of THC, and females’ increased drug sensitivity depends on the stage of their reproductive cycle. This fact has led to our lab to investigate how female sex hormones affect THC sensitivity.
For my thesis, I wanted to determine which female hormone -- estradiol, progesterone, or both, was responsible for females’ increased THC sensitivity. I approached this question by removing the internal source of these hormones, the ovaries, from female rats, and then putting them on a hormone injection regimen to mimic their natural reproductive cycle. I also wanted to examine brain THC sensitivity, because there was evidence that hormones modulate THC’s effect by causing fluctuations in the sites to which THC binds in the brain. Therefore, THC was administered directly into the brain.

After female rats had their ovaries removed and were normalized to a hormone regimen of only oil (placebo), only estradiol, only progesterone, or both estradiol and progesterone, I tested them behaviorally on one of three test days corresponding to different levels of hormones. Rats were injected with either THC or its vehicle directly into the ventricles of the brain and then tested for thermal pain, mechanical pain, locomotor activity, and catalepsy. THC produced pain-relieving and sedative effects on all three test days in all hormone groups. In addition, estradiol significantly increased females’ sensitivity to THC’s effect on the test of mechanical pain.

This study highlights the importance of including females in preclinical biomedical studies: I found that the main female hormone, estradiol, increased THC-induced pain relief on a test of mechanical pain. This shows that sex difference in THC-induced analgesia may be due to an ovarian hormone acting at the level of the brain in females. The results of this study could be used to develop more effective analgesic drugs for women.
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Introduction

Pain is an uncomfortable and often distressing sensation that alerts the body to actual or potential tissue damage. While pain is necessary for survival, it can last long after an injury, causing serious discomfort and decreasing quality of life. Currently one third of the U.S. population suffers from chronic pain (Johannes et al., 2010); thus, improved pain management is an essential topic of further exploration and research.

In the last decade or so, scientific interest in the medicinal potential of cannabinoids, the compounds derived from the marijuana plant (Cannabis sativa), has increased substantially. Cannabis was used for millennia in Central Asia and the Middle East as a sleeping aid, analgesic, anxiolytic, and antidepressant (Iverson, 2008). Numerous modern studies have confirmed that cannabinoids have medical potential, most notably as analgesics. Delta-9-tetrahydrocannabinol (THC), the main psychoactive cannabinoid in cannabis, has been shown to be as effective as codeine in controlling pain (Cambell, 2001). For example, smoked marijuana produced dose-dependent analgesia in healthy humans (Greenwald et al., 2000), and significantly reduced neuropathic pain in HIV patients (Ellis et al., 2009). Synthetic compounds derived from marijuana also produce analgesia in humans. For example, the cannabinoid acid CT3 reduced neuropathic pain with no adverse effects (Karst et al., 2003), and Sativex®, a nasal spray consisting of THC and cannabidiol (another compound found in cannabis), relieved neuropathic
pain without escalation in dose for 52 weeks (Nurmikko et al., 2007). The mechanisms behind the analgesic effects of cannabinoids are not entirely understood, but cannabinoid receptors have been found in areas of the brain, spinal cord and peripheral nervous system that modulate pain (Manzanares, 2006).

The majority of studies that investigate cannabinoids and pain relief use male subjects. In fact, a recent review reported a strong bias towards male research subjects in 8 out of 10 biomedical fields, including neuroscience, pharmacology, endocrinology, and behavioral physiology (Beery and Zucker, 2011). This male bias is detrimental to developing safe and effective medical treatments for women, as it assumes that male and female physiology and endocrinology are the same. Male and female reactions to drugs do differ, and this should be considered especially in studies of pain relief, as women have a 50% greater risk than men for developing painful conditions such as neuropathic pain, musculoskeletal pain, abdominal pain, headache pain, and osteoarthritic pain (Fillingim et al., 2009). Painful disorders such as fibromyalgia, irritable bowel syndrome, multiple sclerosis, and rheumatoid arthritis also are more prevalent in women than in men (Greenspan et al., 2007). The disproportionate incidence of pain conditions in women vs. men suggests that pain-related neurobiology may differ between women vs. men, and that factors such as reproductive hormones may account for sex differences in pain.

Sex differences in various effects of cannabinoids have been observed in animal studies. For example, THC produced a significantly greater effect in female than in male rats using measures of analgesia and motor activity (Tseng and Craft, 2001). Sex hormones are believed
to be responsible for females’ increased sensitivity to cannabinoids. First, estradiol has been shown to increase cannabinoid receptor density in the brains of ovariectomized (OVX) female rats (Rodriguez de Fonseca et al., 1993). Second, the analgesic effects of systemically administered THC were significantly enhanced by administering estradiol to OVX females (Craft and Leitl, 2008). Third, females in late proestrus, the estrous stage following peak estradiol and progesterone levels, showed significantly greater THC-induced analgesia than females in estrus (when hormone levels are lower), and males (Wakley and Craft, 2011). Taken together these studies suggest that ovarian hormones are important modulators of females’ sensitivity to the analgesic effects of THC, and that this hormone modulation may be occurring at the level of the brain.

The cycling female rat can be used as a model for examining possible interactions between hormones and THC in women. Typically, a rat’s ovarian cycle lasts 4-5 days and consists of diestrus, proestrus, and estrus. Estradiol (estradiol benzoate, EB) and progesterone (P4) concentrations peak in the proestrous stage (comparable to the period of ovulation in women); they decline into the estrous stage, reach basal levels in diestrus, and begin to rise again in early proestrus (Freeman, 1988). The fluctuation of these two major ovarian hormones may contribute to the fluctuations in brain sensitivity to THC observed in females.

**Thesis Activity**

For my thesis, I explored ovarian hormone modulation of cannabinoid analgesia by determining which ovarian hormone – EB or P4 or both – is responsible for estrous cycle-related changes in THC sensitivity when THC is administered into the brain.
(intracerebroventricularly, i.c.v.). I removed the major source of ovarian hormones via ovariectomy, and then administered one or both hormones on a cyclic schedule that mimics the rise and fall of these hormones in gonadally intact females. I then conducted behavioral testing by injecting THC or vehicle at three different time points, 8-9 days after surgery.

Methodology

Subjects: Adult (60-90 days old) female Sprague-Dawley rats were used. They were given ad libitum access to food and water except during surgery and testing. Rats were housed in a room with a 12:12 hour light:dark cycle, with the temperature maintained at 21±2°C. Rats were pair-housed until surgeries, and thereafter singly housed to avoid damage to the intracranial implant.

Apparatus: Tail withdrawal analgesia was tested using a 2.5-liter hot water bath kept at 50±1°C. Paw pressure analgesia was tested using an Analgesy-meter. It consisted of a small blunt probe that is placed on the rat’s right hind paw; using a foot pedal to activate an electronic mechanism, the pressure on the rat’s paw is gradually increased at a rate of 48 g/s to a maximum of 990 g. Catalepsy was measured using a bar test; the bar consists of a ring stand with a 1.5-cm diameter horizontal bar placed 12.3 cm above the table surface. Locomotor activity was measured using a photobeam apparatus in which 15 photobeams cross the width of a 20 cm x 40 cm x 23 cm clear cage. Photobeams were spaced 2.5 cm apart and 8 cm above the cage floor.

Surgery: All rats were OVX to eliminate the primary source of EB and P4, and a cannula guide was implanted into one lateral ventricle using a digital stereotaxis. Surgeries were performed
by anesthetizing the rats with ketamine and xylazine at doses of 90 and 10 mg/kg, respectively. Rats recovered for 8-9 days before behavioral testing began.

**Hormone Treatment Regimen:** To mimic the hormone fluctuations seen during a natural 4-day estrous cycle of the rat, OVX rats were injected subcutaneously (s.c.) with 2 µg/0.1 ml EB or safflower oil (the vehicle for the hormones) at 7 am on day 3 and day 7 post-surgery (Surgery day was day 0). 500 µg/0.1 ml P4 or oil was injected s.c. ten hours after EB (at 5 pm) on day 3 and day 7 post-surgery.

**Behavioral Testing:** Behavioral testing occurred starting at either 8 am or 3 pm on day 8, or starting at 8 am on day 9 (see Table). These three time points were chosen because they were expected to capture the peak effect of the ovarian hormones. Tests measured the analgesic and motoric responses to THC. Treatment groups were as follows:

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Behavioral Testing</th>
<th>Day 8, am</th>
<th>Day 8, pm</th>
<th>Day 9, am</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle + vehicle</td>
<td>N=12 vehicle N=16 THC</td>
<td>N=12 vehicle N=16 THC</td>
<td>N=12 vehicle N=16 THC</td>
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<tr>
<td>EB + vehicle</td>
<td>N=12 vehicle N=16 THC</td>
<td>N=12 vehicle N=16 THC</td>
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<tr>
<td>Vehicle + P4</td>
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<td>N=12 vehicle N=16 THC</td>
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<tr>
<td>EB + P4</td>
<td>N=12 vehicle N=16 THC</td>
<td>N=12 vehicle N=16 THC</td>
<td>N=12 vehicle N=16 THC</td>
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**Pretesting Procedure:** Rats were weighed, and a vaginal smear (epithelial cell sample) was obtained from each rat via vaginal lavage. Smears were stained and later examined to
determine the estrous cycle stage (described below). Three baseline values were recorded for the tail withdrawal and paw pressure pain tests (see test descriptions below).

**Testing Procedure:** 100 µg of THC or its vehicle (1:1:8 ethanol:cremaphor:saline) was injected *i.c.v.* in a volume of 5 µl at a rate of 20 µl/min; the injector was held in place for 1 minute following injection to avoid backflow of THC or vehicle out of the cannula. Behavioral tests were conducted starting at 5, 15, 30, 60, 120, and 180 minutes after THC or vehicle injection, unless otherwise specified.

Tail Withdrawal: The tail withdrawal assay was used to measure acute thermal (heat) pain. The rat was loosely restrained in a cloth towel and its tail was submerged about 5 cm into the 50°C water bath. The time it took the rat to withdraw or flick its tail was timed using a stopwatch. A cutoff of 15 seconds was used to avoid tissue damage.

Paw Pressure: The paw pressure assay measured sensitivity to acute mechanical (pressure) pain. The time in seconds that it took for the rat to withdraw its paw or attempt to withdraw its paw from under the weight was measured. This assay was conducted immediately after the tail withdrawal test. The cutoff for this assay was 20 seconds.

Locomotor Activity: The locomotor chambers measure horizontal motor activity by counting the number of photobeams broken in 5 minutes as the rat walks around. The rat was placed into a clean plastic cage that was placed in the locomotor chamber. This assay was conducted at 15, 30, 60, 120, and 180 minutes post-injection, immediately after the paw pressure test.
Catalepsy: The catalepsy bar test also measured motor activity, but more specifically the rat’s
tendency to move when placed in an unnatural position. The rat’s front paws were placed on a
bar set at 12.3 cm above the table surface. The time it took for the rat to either remove both of
its front paws from the bar or jump onto the bar (placing its hind paws on the bar) was
measured using a stopwatch. Catalepsy was measured only at the 15- and 30-minute time
points, immediately after the locomotor activity test, because catalepsy scores increase
significantly with repeated tests (Tseng and Craft, 2001). The cutoff for this assay was 15
seconds.

Post-Testing Procedure: A second vaginal smear was obtained immediately after all behavioral
tests were completed at the last time point (180 minutes post-injection). The rats were then
euthanized via carbon dioxide asphyxiation, and decapitated. Giemsa dye was injected into the
lateral ventricles and the brain was extracted to verify cannula placement. Uteri were
extracted and placed in 10% formalin. Vaginal smear slides were stained with Giemsa dye and
examined to determine estrous stage before and after the time of testing. Proestrus was
identified by the predominance of nucleated epithelial cells, estrus was identified by the
presence of dense sheets of cornified epithelial cells, and diestrus was identified by scattered,
nucleated or cornified cells and leukocytes (diestrus day 1, D1) or a relative paucity of any cells
(diestrus day 2, D2). Proestrus/estrus, also known as “late proestrus”, was determined by the
presence of nucleated cells losing shape and becoming cornified epithelial cells (approximately
half nucleated, half cornified cells in the sample) (Freeman, 1988). After at least 2 weeks in
formalin, uteri were trimmed and weighed as a further confirmation of ovarian hormone effect;
uterine tissue mass is sensitive to ovarian hormone levels (Medlock et al., 1994).
Data Analysis: Only data from rats with accurate cannula placements were included in the analyses. Baseline (no drug) responses were determined for each rat on the tail withdrawal and paw pressure tests as the mean of three pre-injection trials. Individual response latencies following THC or vehicle injection were calculated as % maximum possible effect (%MPE), which is \( \frac{\text{drug latency} - \text{baseline latency}}{\text{cutoff latency} - \text{baseline latency}} \times 100 \).

Tail withdrawal and paw pressure %MPE values, and raw locomotor scores (# of photobeam breaks) also were converted to area-under-the-curve (AUC) values, which were calculated as the area under the time course curves across the 180-min test period, using the trapezoidal rule. AUC values represent total analgesia and locomotor activity during the entire 3-hour testing period, which made it easier to compare analgesia and locomotor activity across days.

AUC values for tail withdrawal, paw pressure, and locomotor activity were analyzed using a 3-way ANOVA: the independent variables were hormone (4 levels), day (3 levels), and THC dose (2 levels). Catalepsy scores at the two time points were averaged for each rat, and then group differences were analyzed using a 3-way ANOVA: the independent variables were hormone (4 levels), day (3 levels), and THC dose (2 levels). Catalepsy scores for rats that exhibited exploratory movement on the catalepsy bar or that were unable to hold onto the bar were also excluded from the catalepsy analysis. Uterine weights were normalized to the rat’s total body weight by dividing the weight of the uterus in grams by the body weight in kilograms and then analyzed using a 3-way ANOVA: the independent variables were hormone (4 levels), day (3 levels), and THC dose (2 levels). A significance level of P ≤ 0.05 was used for all statistical tests. When group differences were observed, Bonferroni’s post hoc test was used.
Results

Tail Withdrawal and Paw Pressure Analgesia. Figure 1 shows AUC values for the tail withdrawal test; AUC values reflect total analgesia over the 3-hour testing period. THC produced analgesia on the tail withdrawal assay in all hormone groups across all three time points (drug: $F(1,313)=244.13, p<.001$). There was no significant hormone modulation of THC’s effect.

Figure 1: i.c.v. THC-induced tail withdrawal analgesia. “O” = oil-treated; “EB” = estradiol-treated; “P4” = progesterone-treated. Vehicle (“Veh”), N=12/hormone group and THC, N=16/hormone group. “Day 8, AM” groups were tested approximately 25 hours after the last oil/EB injection; “Day 8, PM” groups were tested approximately 32 hours after the last oil/EB injection; “Day 9, AM” groups were tested approximately 49 hours after the last oil/EB injection.
Figure 2 shows AUC values for the paw pressure test. THC produced analgesia on the paw pressure assay in all hormone groups across all three time points (drug: $F(1,312)=5.13$, $p<.001$). EB significantly enhanced THC-induced analgesia (drug x EB: $F(1,312)=4.48$, $p=.035$).

Figure 2: *i.c.v.* THC-induced paw pressure analgesia. Details same as in Fig. 1.

**Locomotor Activity.** Figure 3 shows that *i.c.v.* THC suppressed locomotor activity in all hormone groups across all three time points (drug: $F(1,313)=253.77$, $p<.001$). There was a main effect of day ($F(2,313)=8.83$, $p<.001$), with rats tested on Day 8, PM being significantly less active than rats tested on Day 8, AM ($p<.001$) and rats tested on Day 9, AM ($p=.004$). However, there were no significant effects of hormone on THC-induced locomotor suppression.
Catalepsy. Data from 20 rats were removed from analysis due to those rats exhibiting exploratory behavior as opposed to cataleptic behavior, or competing behaviors such as loss of muscle tone or barrel rolling. Data from the remaining rats (~95% of the entire sample) are shown in Figure 4. *i.c.v.* THC produced modest but significant catalepsy (drug: $F(1,293)=78.859, p<.001$). Although it appears that EB increased THC’s cataleptic effect, this effect was not significant (drug x EB: $F(1,293)=3.194, p=.075$).
Figure 4: *I.g.v.* THC-induced catalepsy. 20 rats total were removed due to catalepsy-competing behaviors. Therefore sample sizes for vehicle was N=9-12/hormone group/day and THC N=15-16/ hormone group/day. Other details same as in Fig. 1.

**Uterine Weight**

Figure 5 shows that EB significantly increased uterine weight in OVX females (EB: $F(1,312)=711.22, p<.001$). Neither time point nor THC administration significantly modulated uterine weight.
Vaginal Cytology. Table 1 shows the percent of females in each estrous stage at the time of testing: proestrus (Pro), proestrus/estrus (P/E), estrus (Est) or diestrus (Diest). When treated with only oil or only progesterone, rats were more likely to be in diestrus than any other stages. When treated with estradiol alone or in combination with progesterone, rats were likely to be in either proestrus or proestrus/estrus on Day 8, AM, vs. proestrus/estrus or estrus on Day 8, PM, and Day 9, AM.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Day 8, AM</th>
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<th>Day 8, PM</th>
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<th>Day 9, AM</th>
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<tbody>
<tr>
<td></td>
<td>Pro</td>
<td>P/E</td>
<td>Est</td>
<td>Diest</td>
<td>Pro</td>
<td>P/E</td>
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<tr>
<td>O + O</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>O + P4</td>
<td>4</td>
<td>100</td>
<td>4</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EB + O</td>
<td>36</td>
<td>43</td>
<td>14</td>
<td>7</td>
<td>21</td>
<td>39</td>
</tr>
<tr>
<td>EB + P4</td>
<td>29</td>
<td>43</td>
<td>29</td>
<td>11</td>
<td>36</td>
<td>53</td>
</tr>
</tbody>
</table>
Table 1. Percent of rats in each of 4 vaginal estrous stages at the time of behavioral testing on Day 8, AM, Day 8, PM, and Day 9, AM (approximately 25, 32, and 49 hours after the last oil/estradiol injection, respectively). “Pro” = proestrus; “P/E” = proestrus/estrus (also known as late proestrus); “Est” = estrus; “Diest” = diestrus (day 1 or 2). N=27-28 rats/hormone group/day.

Serum Hormone Levels. Figure 6 shows the levels of EB and P4 in serum (derived from trunk blood samples) of rats that were treated with the same hormone regimen used in this experiment (Wakley et al. in preparation). At the first time point at which behavioral response to THC was tested on Day 8 (8 AM), P4 was elevated and EB had already dropped from its peak. At the second and third time points at which behavioral responses to THC were tested (Day 8, 3 PM and Day 9, 8 AM), both P4 and EB levels had declined.

Figure 6: Serum Estradiol (EB) and Progesterone (P4) Levels. N=4-6/time point.
Discussion

**Hormone Modulation of THC-Induced Analgesia.** This study corroborates previous findings showing that THC produces analgesic and sedative effects when administered *i.c.v.* to gonadally intact female rats (Wakley & Craft, 2011) and when administered to OVX female rats (Craft and Leitl, 2008). The novel finding in the present study is that estradiol, when administered in a cyclical manner to mimic the rat’s natural estrous cycle, increases sensitivity to *i.c.v.* THC-induced analgesia on the paw pressure assay, which measures mechanical pain. This finding corroborates a previous study showing that replacing estradiol chronically (rather than in a cyclic manner) in OVX female rats increased their sensitivity to THC’s analgesic effects when THC was given systemically (Craft & Leitl, 2008).

The previous *i.c.v.* study demonstrating enhanced THC sensitivity in gonadally intact, cycling females showed that it was late proestrous females in particular that had enhanced THC sensitivity on both the tail withdrawal and paw pressure assays (Wakley & Craft, 2011). In contrast, in the present study using OVX females, neither EB nor P4 significantly enhanced the analgesic effects of THC on the tail withdrawal assay of thermal pain. The fact that EB increased *i.c.v.* THC-induced analgesia on the test of mechanical pain but not on the test of thermal pain in this study could be because mechanical pain as measured by the paw pressure assay is modulated at the level of the brain, whereas thermal pain as measured by the tail withdrawal assay may be more dependent on nociceptive processes in the spinal cord. Another possibility regarding why hormones did not modulate THC-induced analgesia on the tail withdrawal assay could be because the ovariectomy surgery disrupts the endocannabinoid system so that it no
longer models that found in an intact cycling female. It is also possible that other ovarian hormones besides EB and P4 such as lutenizing hormone, testosterone, or other estrogens – all of which are disrupted by ovariectomy, but which I didn’t replace – are responsible for females’ cycle-related fluctuation in THC sensitivity on the tail withdrawal test.

**Motoric Effects.** THC suppressed locomotor activity in all hormone groups on all test days. Neither EB nor P4 significantly modulated THC’s motoric effects, which corroborates previous findings of no significant alteration in THC’s motoric effects among females in different estrous stages (Wakley & Craft, 2011; Craft & Leitl, 2008). Considering that the analgesia assays are dependent on motor responses, it is possible that THC-induced analgesia is actually due to motor impairment. However if this were true, patterns seen in motor assays would be reflected in the analgesia assays, which is not the case (i.e., EB did not modulate THC’s sedative effects, but it did modulate THC’s analgesic effects on the paw pressure test).

I did find an effect of day on locomotor activity, with rats tested in the mornings (both vehicle- and THC-treated) more active than rats tested in the afternoon. This finding agrees with other studies showing that locomotor activity in the rat differs in the morning compared to the evening; this circadian rhythm is controlled by the superchiasmatic nucleus in the brain, which is responsible for maintaining daily rhythms (Nováková et al., 1983).

As expected, THC produced mild catalepsy across all hormone groups and time points with no significant hormone modulation, as described previously in our lab (Wakley & Craft, 2011). Catalepsy results were expected to parallel locomotor results considering that they both measure motor behavior. Neither catalepsy nor locomotor activity were modulated by ovarian
hormones, but locomotor activity was affected by time point while catalepsy was not. This suggests that cataleptic behavior is not as susceptible to circadian rhythms as locomotor activity is.

**Vaginal Smears and Uterine Weight.** This study agrees with many previous reports showing that estradiol increases uterine weight in females (e.g., Medlock et al., 1994), and promotes changes in vaginal epithelium cell types (e.g., Freeman et al., 1988). These findings confirm that the cyclic estradiol injection protocol used in the present study was effective at increasing circulating levels of estradiol to proestrus-like levels (Medlock et al., 1994). I expected uterine weight to decrease by Day 9, AM, as EB levels had decreased well before that time point. However, uterine weight of EB-treated rats did not significantly decrease by Day 9, AM, suggesting that EB was still exerting its effects in the uterus at this time point.

Vaginal cytology results showed that when treated with only oil or only progesterone, rats were most likely to be in diestrus (the lowest hormone stage in gonadally intact females), regardless of the time point. When treated with estradiol, alone or in combination with progesterone, rats were likely to be in either proestrus (peak hormone stage in intact females), proestrus/estrus (a stage of declining hormones in intact females), or estrus (low hormone stage in intact females). I expected the majority of rats treated with estradiol alone or in combination with progesterone to be in proestrus on Day 8, AM, proestrus/estrus on Day 8, PM, and estrus on Day 9, AM. The results somewhat agreed with my predictions, in that there was a shift to estrous stages associated with lower hormone levels after Day 8, AM, but not as complete a shift as I expected. Variability is most likely due to individual rats’ metabolism of
estradiol benzoate (a stabilized form of estradiol), which may have taken longer than I anticipated. Thus, results using this model of hormone replacement in OVX female rats generally were consistent with the natural cyclicity of intact females except hormone effects on uterine and vaginal tissue were somewhat prolonged, which agrees with the persistent estradiol enhancement of THC-induced analgesia observed across the day and a half when analgesia was measured. Had I included another time point later on Day 9 or on Day 10, I would expect the estradiol-treated rats to be in diestrus, and the enhancement of THC-induced analgesia to wane.

**Serum Hormone Levels.** The levels of hormone in female rat blood were measured (in an experiment conducted by A. Wakley in our lab) to confirm that each hormone was rising and falling as expected, given the cyclic hormone injection regimen. By the time THC-induced analgesia was first tested (approximately 25 hours after EB injection), EB levels had already dropped from their peak. Testing at this time point was meant to approximate the late proestrous stage, at which our lab previously observed peak sensitivity to THC in gonadally intact females (Wakley et al., 2011). In fact, EB increased THC’s analgesic effect on the paw pressure assay by this first time point, and its effects gradually declined over the next day. The question arises, why is there a delay in EB’s effect? That is, why does enhancement of THC-induced analgesia lag behind peak EB by approximately 1 day? This is most likely because EB exerts its effects on DNA transcription (i.e., EB’s effect is genomic), and it takes some time to increase transcription of CB receptors. This is possibly the mechanism behind EB’s increased effect on THC-induced mechanical analgesia, especially considering that previous studies found that EB increases CB receptor density in the brain (Rodriguez de Fonseca et al., 1993).
**Future Experiments.** This experiment has answered the question of which hormone drives females’ increased sensitivity to THC, but there are further experiments one could perform to elucidate underlying mechanisms and to provide more clinically relevant information about this hormone-drug interaction. For instance, one could replicate this experiment in gonadectomized males with the same hormone regimen of EB and P4, to see if EB still enhanced THC-induced analgesia. If so, it would suggest that EB acts the same in both sexes; if not, it would suggest that males don’t have the capacity to respond to EB as females do, due to classical sexual differentiation. Another useful experiment would be to use a chronic pain model as opposed to the acute pain tests that were used in this experiment. Considering that most patients who go to the doctor for pain have been experiencing it for a while, an experiment using a chronic pain model would be a more clinically relevant test of the hormone-THC interaction. For this experiment one could replicate the surgeries and hormone regimen but also inject them with Complete Freund’s Adjuvant – a killed mycobacterium that elicits immune-mediated swelling, redness and pain in the hindpaw for up to a month -- so that rats would be in a state of chronic pain, and then test whether EB still increases THC’s analgesic effect.

**Conclusion**

THC has been shown to produce greater analgesia in female compared to male rats (Tseng & Craft, 2001). Females’ greater sensitivity to THC depends on their reproductive cycle stage: late proestrous (sexually receptive) females show greater THC-induced analgesia than do estrous females, and males (Wakley & Craft, 2011). This previous finding suggested that
ovarian hormones modulate the analgesic effect of THC in females. Furthermore, cannabinoid receptors have been found in areas of the brain that modulate pain (Lichtman et al., 1996), and cannabinoid receptor density has been shown to fluctuate as a function of estrous stage (Rodriguez de Fonseca et al., 1993), suggesting that hormone modulation of THC’s effect is occurring in the brain. The present results support the hypothesis that the ovarian hormone estradiol acts in the brain to increase females’ sensitivity to the analgesic effects of THC.

The aim of the present study was to determine which ovarian hormone, estradiol, progesterone or both, is responsible for reproductive cycle-related changes in brain THC sensitivity, and at which time point after peak hormone levels females are the most THC-sensitive. Estradiol enhanced THC-induced analgesia on the paw pressure test of mechanical pain, but neither estradiol nor progesterone modulated THC-induced analgesia on the tail withdrawal assay of thermal pain, or consistently modulated the motoric effects of THC.

This experiment is one of the few preclinical studies to examine the hormonal mechanisms behind sex differences in drug sensitivity. It is becoming increasingly clear that males and females respond to a variety of pharmacological agents differently, and if we are able to better understand these differences, we can take advantage of them to create more effective drugs that are tailored to one’s sex and/or hormonal state. For example, in this study I found that the main female ovarian hormone, estradiol, is responsible for increasing THC-induced analgesia against mechanical pain. Perhaps this finding can be exploited and tested further, eventually leading to the development of an analgesic specifically for women, which
could be effective against mechanical pain such as occurs in osteoporosis, broken limbs, and back and neck pain.
References


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Thesis Presented Spring 2013

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