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Effects of Δ9-THC and cannabidiol vapor inhalation in male and female rats

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Abstract

Rationale: Previous studies report sex differences in some, but not all, responses to cannabinoids in rats. The majority of studies use parenteral injection, however most human use is via smoke inhalation and, increasingly, vapor inhalation.

Objectives: To compare thermoregulatory and locomotor responses to inhaled Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD) and their combination using an e-cigarette based model in male and female rats.

Methods: Male and female Wistar rats were implanted with radiotelemetry devices for the assessment of body temperature and locomotor activity. Animals were then exposed to THC or CBD vapor using a propylene glycol (PG) vehicle. THC dose was adjusted via the concentration in the vehicle (12.5-200 mg/mL) and the CBD (100, 400 mg/mL) dose was also adjusted by varying the inhalation duration (10-40 minutes). Anti-nociception was evaluated using a tail-withdrawal assay following vapor inhalation. Plasma samples obtained following inhalation in different groups of rats were compared for THC content.

Results: THC inhalation reduced body temperature and increased tail-withdrawal latency in both sexes equivalently and in a concentration-dependent manner. Female temperature, activity and tail-withdrawal responses to THC did not differ between estrus and diestrus. CBD inhalation alone induced modest hypothermia and suppressed locomotor activity in both males and females. Co-administration of THC with CBD, in a 1:4 ratio, significantly decreased temperature and activity in an approximately additive manner and to similar extent in each sex. Plasma THC varied with the concentration in the PG vehicle but did not differ across rat sex.

Conclusion: In summary the inhalation of THC or CBD, alone and in combination, produces approximately equivalent effects in male and female rats. This confirms the efficacy of the e-cigarette based method of THC delivery in female rats.

Keywords

e-cigarette; cannabidiol; hypothermia; locomotor activity; sex differences

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Introduction

Human ingestion of *Cannabis sativa* is presumably reinforced by the effects of the phytocannabinoid Δ^9-tetrahydrocannabinol (THC), the major psychoactive component of recreational cannabis (Burgdorf et al. 2011; ElSohly et al. 2016). There may be sex differences in the effects of THC since women reported significantly more dizziness, accompanied by a greater drop in mean arterial pressure (Mathew et al. 2003), and less tachycardia than men after smoking cannabis (Cocchetto et al. 1981). In addition marijuana smoking women exhibit greater deficits in visuospatial memory than their male counterparts during abstinence (Pope et al. 1997). Similarly, THC has been reported to be more potent in female compared with male rodents in producing anti-nociception (Tseng and Craft 2001), hypothermia (Borgen et al. 1973; Wiley et al. 2007), and motoric effects (Cohn et al. 1972; Tseng and Craft 2001). On the other hand, no apparent human sex differences were found in subjective intoxication or plasma levels of THC after smoking marijuana (Mathew et al. 2003; Wall et al. 1983) or in the effects of THC on impulsivity (McDonald et al. 2003). Male rodents may be more sensitive than females to the hyperphagic effect of cannabinoid agonists (Diaz et al. 2009) and female rats may be more sensitive than males to the adverse effects of escalating adolescent THC exposure on emotional behavior and stress reactivity in adulthood (Rubino et al. 2008). Thus it remains of significant interest to determine any sex differences/similarities in the effects of THC.

Humans typically smoke cannabis, and more recently are turning to noncombusted inhalation techniques (Morean et al. 2015), yet almost all preclinical studies of sex differences with cannabinoids have involved systemic injection such as intraperitoneal (Wiley et al. 2007) or intravenous (Martin et al. 1991) administration. The route of administration of cannabinoids, and particularly THC, can cause variability in the pharmacokinetic, pharmacodynamic and behavioral effects in humans and non-human animals (Fried and Nieman 1973; Manwell et al. 2014; Naef et al. 2004; Niyuhire et al. 2007; Wilson et al. 2002). Parenteral administration of cannabinoid agonists suppresses spontaneous activity, decreases nociception, induces hypothermia, and increases catalepsy in rodents of both sexes (Compton et al. 1993; Wiley et al. 2007) thus these measures predominate in rodent models of the effects of THC. Inhalation delivery of THC with a custom metered-dose inhaler confirmed comparable degrees of anti-nociception, hypothermia, and catalepsy in mice exposed to the THC aerosol (Lichtman et al. 2000; Wilson et al. 2002) compared with parenteral injection of THC. In addition, THC levels in the blood and brains of mice were similar following either marijuana smoke inhalation exposure or intravenous injection of THC (Lichtman et al. 2001).

An additional phytocannabinoid of recent interest (see (Boggs et al. 2018) for review), cannabidiol (CBD), does not activate the CB_1 and CB_2 receptors (Pertwee 2008) but has shown activity in opposition to the effects of CB_1/CB_2 receptor agonists in some findings (Morgan et al. 2010; Radwan et al. 2009; Wright et al. 2013). On the other hand, CBD does not oppose, and may enhance, hypothermia caused by THC, i.p. when administered in 1:1-1:3 THC:CBD ratios in rats (Taffe et al. 2015). It remains unknown how CBD may alter the effects of THC when administered by inhalation in laboratory species.

Our recent study (Nguyen et al. 2016b) validated a new method of rat cannabinoid inhalation to model current human use of e-cigarettes to administer cannabinoids. That prior study included a limited sex comparison that appeared to demonstrate greater female hypothermia following THC inhalation. Thus a new study was designed to more fully compare the effects of THC inhalation between male and female rats on nociception, thermoregulation, spontaneous activity and plasma THC levels.

Materials and methods

Subjects:

Age-matched groups of Wistar rats (Charles River, NC, USA) were housed in humidity and temperature-controlled (23±1 °C) vivaria on 12:12 hour light:dark cycles with *ad libitum* access to food and water in their home cages. Animals entered the laboratory at ~10 weeks of age. All procedures were conducted in the animals' dark (active) cycle under protocols approved by the Institutional Care and Use Committee of The Scripps Research Institute and in a manner consistent with the Guide for the Care and Use of Laboratory Animals (National Research Council (U.S.). Committee for the Update of the Guide for the Care and Use of Laboratory Animals. et al. 2011).

Radiotelemetry:

Male (N=8) and female (N=8) Wistar rats were anesthetized with an isoflurane/oxygen vapor mixture (isoflurane 5% induction, 1-3% maintenance) and sterile radiotelemetry transmitters (Data Sciences International, St. Paul, MN; TA-F40) were implanted in the abdominal cavity through an incision along the abdominal midline posterior to the xyphoid space as previously described (Javadi-Paydar et al. 2018).

Activity and temperature responses were evaluated in clean standard plastic home cages (thin layer of bedding) in a dark testing room, separate from the vivarium, during the (vivarium) dark cycle. In some experiments (detailed below) animals were habituated in recording chambers, moved to the vapor inhalation chamber for the exposure and then returned to the separate recording chamber. In other experiments, telemetry plates under the vapor inhalation chambers were used throughout the recording. Radiotelemetry transmissions were collected via telemetry receiver plates placed under the cages as previously described (Nguyen et al. 2016b).

Inhalation Apparatus:

Sealed exposure chambers were modified from the 259mm X 234mm X 209mm Allentown, Inc (Allentown, NJ) rat cage to regulate airflow and the delivery of vaporized drug to rats using e-cigarette cartridges (Protank 3 Atomizer, MT32 coil operating at 2.2 ohms, by Kanger Tech; Shenzhen Kanger Technology Co.,LTD; Fuyong Town, Shenzhen, China) as has been previously described (Nguyen et al. 2016a; Nguyen et al. 2016b). An e-vape controller (Model SSV-1; 3.3 volts; La Jolla Alcohol Research, Inc, La Jolla, CA, USA) was triggered to deliver the scheduled series of puffs by a computerized controller designed by the equipment manufacturer (Control Cube 1; La Jolla Alcohol Research, Inc, La Jolla, CA, USA). The chamber air was vacuum controlled by a chamber exhaust valve (i.e., a "pull"

system) to flow room ambient air through an intake valve at ~1 L per minute. This also functioned to ensure that vapor entered the chamber on each device triggering event. The vapor stream was integrated with the ambient air stream once triggered.

Nociception Assay:

Tail withdrawal anti-nociception was assessed 60 minutes after the initiation of inhalation using a water bath (Bransonic® CPXH Ultrasonic Baths, Danbury, CT) maintained at 48, 50 or 52 °C. Three different temperature conditions were evaluated in the event of any non-linearities in the effect of THC across rat sex. The latency to withdraw the tail was measured using a stopwatch and a cutoff of 15 seconds was used to avoid possible tissue damage (Wakley et al. 2014a).

Drugs:

Rats were exposed to vapor derived from Δ^9-tetrahydrocannabinol (THC; 12.5, 25, 50, 100, 200 mg/mL) or cannabidiol (100, 400 mg/mL) dissolved in a propylene glycol (PG) vehicle. The ethanolic THC stock was aliquoted in the appropriate volume, the ethanol evaporated off and the THC was then dissolved in the PG to achieve target concentrations. Four 10-s vapor puffs were delivered with 2-s intervals every 5 minutes, which resulted in use of approximately 0.125 mL in a 40 minutes exposure session (Nguyen et al. 2016b). The vacuum was turned off for the 4 minute, 12 second interval between vapor deliveries and then turned up to ~3-5 L/minutes at the conclusion of sessions for ~5 minutes to facilitate complete chamber clearance for subject removal. THC was suspended in a vehicle of 95% ethanol, Cremophor EL (Spectrum Chemical MFG Corp., Gardena CA) and saline (Bacteriostatic 0.9% sodium chloride injection, USP; Baxter Healthcare Corp., Deerfield, IL) in a 1:1:8 ratio, for intraperitoneal injection. The THC was provided by the U.S. National Institute on Drug Abuse Drug Supply Program and cannabidiol was obtained from Cayman Chemical (Ann Arbor, MI).

Determination of estrous stage:

The stage of the estrous cycle was determined using interpretation of vaginal cytology. Unstained vaginal smears were viewed immediately upon collection via pipet lavage. Proestrus was indicated when cells were predominantly nucleated epithelial cells. A predominance of cornified epithelial cells classified estrus. Metestrus was recognized by scattered, nucleated or cornified epithelial cells and leukocytes, and diestrus was classified by classic leukocytes in combination with various larger round epithelial cells. (Freeman 1988; Goldman et al. 2007). Vaginal samples were taken in the evening prior to the day of experiment to facilitate planning the dosing and a second smear was obtained ~ 1 hour before THC inhalation to confirm. The estrous and diestrous stages were selected for this experiment because a previous study reported the largest stage-related differential effect of 5 mg/kg THC in estrus versus diestrus in gonadally intact, cycling females (Craft and Leitl 2008).

Plasma THC/CBD analysis:

For single time point studies blood samples were collected (~500-1000 ul) via jugular needle insertion under anesthesia with an isoflurane/oxygen vapor mixture (isoflurane 5% induction, 1-3% maintenance). For serial blood sampling (200 ul; 35, 60, 120 and 240 minutes after vapor initiation) rats were prepared with chronic intravenous catheters as previously described (Aarde et al. 2017). Catheter function was assessed prior to starting a series of experiments and only animals with a functional catheter were used; a two week recovery interval was imposed prior to any further experimentation for multiple-sampling procedures. Plasma THC content was quantified using fast liquid chromatography/mass spectrometry (LC/MS) adapted from (Irimia et al. 2015; Lacroix and Saussereau 2012; Nguyen et al. 2017). Fifty μL of plasma were mixed with 50 μL of deuterated internal standard (100ng/mL CBD-d3 and THC-d3; Cerilliant), and cannabinoids were extracted into 300 μL acetonitrile and 600 μL of chloroform and then dried. Samples were reconstituted in 100 μL of an acetonitrile/methanol/water (2:1:1) mixture. Separation was performed on an Agilent LC1100 using an Eclipse XDB-C18 column (3.5um, 2.1mm x 100mm) using gradient elution with water and methanol, both with 0.2 % formic acid (300 μL/min; 73-90%). Cannabinoids were quantified using an Agilent MSD6180 single quadrupole using electrospray ionization and selected ion monitoring [CBD (m/z=315.2), CBD-d3 (m/z=316.7), THC (m/z=313.7) and THC-d3 (m/z=316.7)]. Calibration curves were conducted for each assay at a concentration range of 0-200 ng/mL and observed correlation coefficients were 0.999.

Experiments:

A minimum 7 day interval separated all active THC dosing for a given individual throughout the following experimental conditions which are listed in the order conducted (Figure 1).

Experiment 1, Effect of Vapor Inhalation of THC (12.5-100 mg/mL) in Male and Female Rats: The first experiment was conducted in experimentally naïve animals to determine the effect of altering THC concentration in the PG. Animals were placed in individual telemetry recording cages for at least 30 minutes of habituation prior to the start of inhalation; this initial telemetry interval was used as the pre-treatment baseline. Rats were transferred in pairs to the chambers for vapor exposure and thereafter returned to their recording cages (this approach was also used for Experiments 2-4). In this experiment, telemetry recording was conducted following dosing conditions of PG or THC (12.5, 25, 50, 100 mg/mL) with the order counter-balanced by pairs. Recording continued for up to 4 hours after the start of vapor inhalation. Subsequent experiments were conducted in the same groups of male and female animals with the test conditions counter-balanced within experiment as described below.

Experiment 2, Impact of Estrous Phase on the Effects of Inhaled THC: After Experiment 1, the female telemetry group was recorded during estrus and diestrus by determining phase daily, and only conducting an experiment that day if the animal was in the target phase. First the female rats were recorded following 30 minutes of inhalation of THC 50 mg/mL (versus PG) in estrus and diestrus for four total treatment conditions. They were next assessed following 40 minutes of inhalation of THC 25 mg/mL versus PG to further

explore the potentially more rapid recovery in estrus identified after the 50 mg/mL THC inhalation.

Experiment 3, Vapor Inhalation of THC with Cannabidiol (CBD): After Experiment 1, the male group was evaluated for responses to 30 min of inhalation of PG, CBD (100 mg/mL), THC (200 mg/mL) or the combination in counter-balanced order.

Experiment 4, Cannabidiol Duration/Response: Next, both male and female groups were evaluated for responses to CBD inhalation. In this case, the dosing conditions were CBD (100 mg/mL) for three different inhalation durations (10, 20, 40 minutes), or PG for a 20 minute duration, in a counter-balanced order.

Experiment 5, Effects of Threshold THC (25 mg/mL) + CBD (100 mg/mL) Combination: After Experiment 4, telemetry recordings were collected for male and female rat groups following exposure to PG, CBD (100 mg/mL), THC (25 mg/mL) or the combination for 30 minutes. For this study, the telemetry recording was conducted throughout vapor inhalation, thus animals were dosed singly and all recordings were from rats in the inhalation chamber. All 8 animals within the male/female groups were run simultaneously (different inhalation conditions) on a given day and the male and female groups were evaluated on different days.

Experiment 6, Anti-nociceptive Effect of THC: Nociception was assessed in both male and female groups 60 minutes after the initiation of inhalation of PG or THC (100 mg/mL) for 30 minutes. Nociception was assessed for the two inhalation condition under three different water bath temperatures with all six conditions evaluated in a counter-balanced order. THC exposure was no more frequently than weekly and there were at least two days between any nociception assessments. Following this study, the female group was evaluated for nociception after inhalation of PG or THC (100 mg/mL) for 30 minutes in estrus and diestrus, in counter-balanced order.

Experiment 7, Effects of High Dose THC (100 mg/mL) + CBD (400 mg/mL) Combination: Finally, telemetry recordings for both male and female rats were obtained following inhalation of PG, CBD (400 mg/mL), THC (100 mg/mL) or the combination for 30 minutes in counter-balanced dosing order. This represented the same 1:4 THC:CBD ratio evaluated in Experiment 5 but at a higher overall dose. Animals were dosed and recorded as described in Experiment 5.

Experiment 8, Pharmacokinetics: Pharmacokinetic experiments were conducted in additional groups of male and female Wistar rats. The first group of male (N=8) and female (N=8) Wistar rats were 20 weeks of age at the start of this study. These rats arrived in the lab as a cohort and were previously implanted with jugular catheters, for methods see (Aarde et al. 2017); one male and one female rat did not survive catheter surgery. Intact rats with patent catheters at 20 weeks of age (2 out of 7 male and 3 out of 7 female rats) were used for serial plasma collection at 35, 60, 120 and 240 minutes after the start of inhalation of THC Vapor (100 mg/mL, 30 min). Then, the same total group of rats (7 per sex; male mean weight 424.1 g SEM 17.0, female mean weight 256.2 g SEM 5.1) were exposed to 30 min of

inhalation of THC in three concentrations (25, 100, 200 mg/mL) with single jugular blood withdrawals (~0.5 mL) obtained post-session (i.e., 35 minutes after the start of inhalation) and at 60 minutes after the start of inhalation for 6 total sessions. THC exposure was conducted no more frequently than weekly for single blood withdrawals and biweekly for multiple blood draws (Diehl et al. 2001). A second group of male and female Wistar rats, 38 weeks of age at the start of this study, were used for determining plasma levels after i.p. injection of THC. These animals arrived in the lab as a cohort (N=8 per sex) and were previously exposed to doses of oxycodone, heroin, methadone and buprenorphine via vapor inhalation and parenteral injection over an interval of 14 weeks for anti-nociception experiments (one male rat died after an oxycodone challenge thus N=7 for this study). The PK study was conducted starting 12 weeks after the end of the prior studies at 38 weeks of age (male mean weight 706.3 g SEM 22.8; female mean weight 337.9 g SEM 13.6). The rats were divided into two independent groups (3-4 rats per sex) and were injected with one of two THC doses (3, 10 mg/kg, i.p.) followed by a single blood collection obtained 30 minutes post-injection.

Data Analysis

Temperature and activity rate (counts per minute) were collected via the radiotelemetry system on a 5-minute schedule and analyzed in 30 minute averages (in the graphs the time point refers to the ending time, i.e. 60 = average of 35-60 minute samples). Activity counts with the Data Sciences system reflect a consistent, but arbitrary value of movement of the transmitter relative to the receptive field of the receiver plate. Any missing temperature values were interpolated from preceding and succeeding values, this amounted to less than 10% of data points. Telemetry data were analyzed with Analysis of Variance (ANOVA) with repeated measures factors for the Drug Inhalation Condition, the Time post-initiation of vapor and estrous phase where relevant. Tail-withdrawal latencies were analyzed by ANOVA with repeated measures factors of Drug Treatment Condition and Water Bath temperature and between-groups factor of sex. Plasma levels of THC were analyzed with between-groups ANOVA due to the design with factors for Time post-initiation of inhalation, drug Concentration in the PG and/or sex, as described in the Results for specific experiments. Any significant effects within group were followed with post-hoc analysis using Tukey correction for all multi-level, and Sidak correction for any two-level, comparisons. All analysis used Prism 7 for Windows (v. 7.03; GraphPad Software, Inc, San Diego CA).

Results

Experiment 1: Effect of Vapor Inhalation of THC (12.5-100 mg/mL) in Male and Female Rats:

Females: The *temperature* of female rats was decreased by THC inhalation in a concentration dependent manner (Figure 2). Temperature was significantly different (Table 1) from the baseline after inhalation of 25 mg/mL, 50 mg/mL or 100 mg/mL THC but not after the 12.5 mg/mL THC or PG inhalation. Temperature did not differ compared with PG in the 12.5 or 25 mg/mL concentrations, but the post-hoc test confirmed significant differences from vehicle at a corresponding time point after 50 mg/mL or 100 mg/mL THC inhalation. Temperature was also significantly different following inhalation of 50 mg/mL

versus 100 mg/mL. In addition, the body temperature was significantly lower compared with the 12.5 mg/mL condition following 50 mg/mL or 100 mg/mL and lower than the 25 mg/mL following 50 mg/mL or 100 mg/mL. The *activity* of female rats was significantly different across the duration of the recording session but this was not affected by vapor inhalation condition.

Males: The *temperature* of male rats was also decreased by THC inhalation (Figure 2) in a concentration dependent manner. The temperature was significantly different (Table 1) from the baseline after inhalation of vapor from the 50 mg/mL or 100 mg/mL concentration but not after the 12.5 mg/mL, 25 mg/mL or vehicle inhalation conditions. Temperature differed after inhalation of 50 mg/mL or 100 mg/mL THC, but not the lower concentrations, compared with the vehicle condition at a corresponding time point after the initiation of vapor. In addition, the temperature of male rats was significantly lower compared with the 12.5 or 25 mg/mL conditions following 50 mg/mLor 100 mg/mL THC inhalation. The *activity* of male rats changed across the recording sessions and was also changed by inhalation condition. The marginal mean analysis confirmed that significantly less activity was observed after 50 mg/mL or 100 mg/mL THC inhalation compared with the vehicle.

Follow-up analysis compared the impact of vapor inhalation in the 60 min time point compared with the baseline across groups to directly compare the sexes. This three-way ANOVA first confirmed that temperature was significantly affected by inhalation Dose condition [$F(4, 4) = 31.95$; $P<0.0001$], by Time point [$F(1, 4) = 21.15$; $P<0.0001$] and by the interaction of Dose with Time [$F(4, 4) = 36.09$; $P<0.0001$] but not by Sex or the interaction of Sex with any other factor. A similar analysis failed to confirm any main or interacting effects of Sex, Dosing condition or Time on activity rate.

Experiment 2: Impact of Estrous Phase on the Effects of Inhaled THC

THC (50 mg/mL; 30 minutes): Mean body temperature was again significantly lowered (Table 2) by THC inhalation (Figure 3) and in this case temperature was significantly lower compared with the baseline value after inhalation of 50 mg/mL THC during estrus and diestrus. The post-hoc test also confirmed that temperature differed from the corresponding time point after vehicle inhalation following 50 mg/mL THC during either estrus or diestrus. Temperature only differed between estrus and diestrus 240 minutes after the start of inhalation.

In this study the activity rate was lower after THC inhalation compared with the vehicle condition in both estrus and diestrus (Table 2). The post-hoc test confirmed that activity was significantly different from the baseline, and from corresponding time points after vehicle inhalation, following the inhalation of 50 mg/mL THC in estrus and diestrus. Activity was significantly different between estrus and diestrus 240 minutes after the start of inhalation of THC 50 mg/mL THC.

THC (25 mg/mL; 40 minutes): A total of N=6 rats completed the estrus evaluation and N=7 completed the diestrus evaluation due to scheduling constraints (Figure 4). The rats' body temperature was again decreased by THC inhalation relative to vehicle inhalation. The post-hoc test confirmed that temperature was significantly different from the baseline value

after inhalation of 25 mg/mL THC in both estrus and diestrus. Temperature also differed from the corresponding time point after vehicle inhalation when 25 mg/mL THC was inhaled during either estrous or diestrous phases. Finally, temperature was not different between estrus and diestrus within either inhalation condition.

Locomotor activity was significantly different from the baseline value after 40 minutes exposure to 25 mg/mL THC when rats were in diestrus but activity rate was not significantly different between estrus and diestrus after exposure to THC 25 mg/mL.

Experiment 3: Vapor Inhalation of THC with Cannabidiol (CBD):

Males: Males were next evaluated in conditions of vehicle versus CBD (100 mg/mL) for 30 minutes in counter-balanced order and then vehicle, THC (200 mg/mL) and THC (200 mg/mL) + CBD (100 mg/mL) for 30 minutes in counter-balanced order. Preliminary analysis identified no differences between the first and second vehicle conditions and thus the second one was used for analysis purposes. Body temperature was decreased (Table 3) by cannabinoid inhalation (Figure 5) and the post-hoc test confirmed that body temperature was significantly different from the baseline after vehicle, CBD 100 mg/mL, THC 200 mg/mL, and after THC 200 mg/mL + CBD 100 mg/mL inhalation. Correspondingly, body temperature was significantly lower than in the corresponding time point after vehicle inhalation following CBD 100 mg/mL, THC 200 mg/mL, and THC 200 mg/mL + CBD 100 mg/mL inhalation. In addition, significant differences from the CBD 100 mg/mL condition were confirmed for THC 200 mg/mL and THC 200 mg/mL + CBD 100 mg/mL inhalation. Body temperature was never different between the THC 200 mg/mL and THC 200 mg/mL + CBD 100 mg/mL inhalation conditions.

Activity rate was also altered by drug inhalation and the post-hoc test confirmed that activity was significantly different from the baseline after CBD 100 mg/mL, THC 200 mg/mL, and after THC 200 mg/mL + CBD 100 mg/mL inhalation. Activity was also significantly lower compared with the corresponding time point after vehicle inhalation condition following CBD 100 mg/mL, THC 200 mg/mL, and THC 200 mg/mL + CBD 100 mg/mL inhalation. A significant difference from the CBD 100 mg/mL condition was confirmed for the THC 200 mg/mL + CBD 100 mg/mL condition but activity was never significantly different between the THC 200 mg/mL and THC 200 mg/mL + CBD 100 mg/mL inhalation conditions.

Experiment 4: Cannabidiol Duration/Response:

Females: The *temperature* of female rats was decreased by CBD (Figure 6) inhalation and the post-hoc test confirmed that temperature was significantly lower compared with the baseline following inhalation of CBD for 20 minutes and for 40 minutes; see Table 4 for statistical details. Temperature was also significantly lower compared with the vehicle condition following inhalation of CBD for 40 minutes (60-90 minutes after the start of inhalation). The *activity* rate was altered by CBD inhalation and the post-hoc test confirmed that activity was significantly lower compared with the baseline following inhalation of CBD for 20 minutes or 40 minutes. Activity rate was also significantly lower, compared with the corresponding time point after vehicle inhalation, following inhalation of CBD for 10, 20 or 40 minutes.

Males: The *temperature* of male rats (Figure 6) was likewise altered (Table 4) by CBD. The post-hoc test confirmed that temperature was significantly lower compared with the baseline following inhalation of vehicle as well as after inhalation of CBD for 40 minutes. Temperature was also significantly lower compared with the vehicle condition following inhalation of CBD for 20 minutes or 40 minutes and lower compared with the CBD 10 minute inhalation condition following inhalation of CBD for 20 or 40 minutes. Male rat *activity* rate was also affected by vapor inhalation and the post-hoc of the marginal means confirmed that activity was lower in the 20 minute CBD inhalation condition compared with vehicle inhalation.

Experiment 5: Effects of Threshold THC (25 mg/mL) + CBD (100 mg/mL) Combination

Females: The *temperature* of female rats was decreased by drug inhalation (Table 5) and the post-hoc test confirmed that body temperature was significantly different from the baseline after vehicle, CBD 100 mg/mL, THC 25 mg/mL, and THC 25 mg/mL + CBD 100 mg/mL inhalation (Figure 7). Correspondingly, body temperature was significantly lower compared with the corresponding timepoint after vehicle inhalation following CBD 100 mg/mL, THC 25 mg/mL, and after THC 25 mg/mL + CBD 100 mg/mL inhalation. In addition, significant differences from the CBD 100 mg/mL condition were confirmed for THC 25 mg/mL and for THC 25 mg/mL + CBD 100 mg/mL inhalation. Body temperature also differed between the THC 25 mg/mL and THC 25 mg/mL + CBD 100 mg/mL inhalation conditions.

Activity rate was significantly different from baseline after vehicle, CBD 100 mg/mL, THC 25 mg/mL, and THC 25 mg/mL + CBD 100 mg/mL inhalation. Activity was significantly lower than the vehicle inhalation condition following CBD 100 mg/mL and THC 25 mg/mL + CBD 100 mg/mL inhalation. A significant difference from the CBD 100 mg/mL condition was confirmed for THC 25 mg/mL 60 minutes after the start of inhalation and activity was also significantly different between the THC 25 mg/mL and THC 25 mg/mL + CBD 100 mg/mL conditions, again 60 minutes after the start of inhalation.

Males: Male rat *temperature* (Figure 7) was significantly different (Table 5) from the baseline after vehicle, CBD 100 mg/mL, THC 25 mg/mL, and THC 25 mg/mL + CBD 100 mg/mL inhalation. Correspondingly, body temperature was significantly lower than the same time point after vehicle inhalation following CBD 100 mg/mL, THC 25 mg/mL, and after THC 25 mg/mL + CBD 100 mg/mL inhalation. In addition, significant differences from the CBD 100 mg/mL condition were confirmed for THC 25 mg/mL + CBD 100 mg/mL inhalation and temperature was also different after the THC 25 mg/mL and THC 25 mg/mL + CBD 100 mg/mL inhalation.

The *activity* rate of male rats was significantly different from the baseline after vehicle, CBD 100 mg/mL, THC 25 mg/mL, and THC 25 mg/mL + CBD 100 mg/mL inhalation. Activity was significantly lower than the vehicle inhalation condition following CBD 100 mg/mL, THC 25 mg/mL, and THC 25 mg/mL + CBD 100 mg/mL inhalation. There was a significant difference between activity in the CBD 100 mg/mL and THC 25 mg/mL + CBD 100 mg/mL

conditions, 60 minutes after the start of inhalation. Activity also differed significantly between the THC 25 mg/mL and THC 25 mg/mL + CBD 100 mg/mL conditions.

Experiment 7: Effects of High Dose THC (100 mg/mL) + CBD (400 mg/mL) Combination

The initial THC+CBD combination in male rats was conducted using a higher THC concentration (200 mg/mL) and a 2:1 THC:CBD ratio and the second study used a 1:4 THC:CBD ratio at lower concentrations. To further explore potentially additive effects, male and female rats completed a study of the effects of inhalation of PG, CBD (400 mg/mL), THC (100 mg/mL) versus THC (100 mg/mL) + CBD (400 mg/mL) for 30 minutes in counter-balanced order.

Females: The *temperature* of female rats was decreased by drug inhalation (Figure 8) and the post-hoc test confirmed that body temperature was significantly different from the baseline after vehicle, CBD 400 mg/mL, THC 100 mg/mL, and THC 100 mg/mL + CBD 400 mg/mL inhalation; see Table 6 for statistical details. Correspondingly, body temperature was significantly lower than the corresponding time point after vehicle inhalation following CBD 400 mg/mL, THC 100 mg/mL, and THC 100 mg/mL + CBD 400 mg/mL inhalation. In addition, significant differences from the CBD 400 mg/mL condition were confirmed for the THC 100 mg/mL and for the THC 100 mg/mL + CBD 400 mg/mL conditions. Body temperature was also different between the THC 100 mg/mL and THC 100 mg/mL + CBD 400 mg/mL inhalation conditions.

The *activity* rate was likewise changed after drug inhalation. Activity was significantly different than in the corresponding time point after vehicle inhalation following CBD 400 mg/mL and after THC 100 mg/mL + CBD 400 mg/mL inhalation. A significant difference from the CBD 400 mg/mL condition was confirmed for THC 100 mg/mL+ CBD 400 mg/mL inhalation and activity was also significantly different between the THC 100 mg/mL and THC 100 mg/mL + CBD 400 mg/mL inhalation conditions.

Males: The *temperature* of male rats was changed (Table 6) by drug inhalation and the post-hoc test confirmed that body temperature was significantly different from the baseline after vehicle, CBD 400 mg/mL, THC 100 mg/mL, and THC 100 mg/mL + CBD 400 mg/mL inhalation(Figure 8). Correspondingly, body temperature was significantly lower than the corresponding time point after vehicle inhalation following CBD 400 mg/mL, THC 100 mg/mL, and THC 100 mg/mL + CBD 400 mg/mL inhalation. In addition, significant differences from the CBD 400 mg/mL condition were confirmed for THC 100 mg/mL and THC 100 mg/mL + CBD 400 mg/mL inhalation. Body temperature was also different between the THC 100 mg/mL and THC 100 mg/mL + CBD 400 mg/mL conditions. The *activity* rate was also changed and the marginal mean post-hoc test confirmed that the activity rate was significantly higher than all other time points, across vapor conditions, 30 min after the start of vapor, and significantly lower than baseline activity 150-240 minutes after the start of inhalation.

No Alteration in the Effect of THC Across Repeated Experimentation

To assess possible tolerance or sensitization of the response to the inhalation of THC across dosing conditions, a further analysis compared the temperature response to 100 mg/mL THC (for 30 minutes) in the first experiment with the same inhalation conditions conducted during the high-dose combination study (Figure 9) across rat sex. The analysis confirmed no differences in the initial male and female body temperature responses across time points. The female temperature at Time 2 (assessed during Weeks 30-35) returned to baseline more slowly with significant differences from each other group/time 150-180 min post-initiation and from the males at Time 1 (assessed during Weeks 3-8) at 150-210 minutes post-initiation. The 2-way ANOVA confirmed that body temperature was significantly affected by sex/dose condition [$F (3, 28) = 3.51$; $P < 0.05$], by Time Post-Initiation [$F (6, 168) = 35.10$; $P < 0.0001$] and by the interaction of factors [$F (18, 168) = 2.39$; $P < 0.01$]. The Tukey post-hoc test further confirmed that temperature was significantly different from males at Time 1 at 150-210 minutes and from males at Time 2 at 150-180 minutes. The mean body weight of the females was 61% of that of the males at Time 1 and 52% at Time 2. Female weight increased by 43% from Time 1 to Time 2 and the males increased by 66%.

Experiment 6: Anti-nociceptive Effect of THC

THC inhalation decreased sensitivity to a noxious stimulus in both males and female rats and this effect was similar across different water bath temperature condition (Figure 10). The initial 3-way ANOVA confirmed that tail withdrawal latency was significantly affected by sex, THC/PG conditionand water bath temperature and also by the interaction of THC treatment condition and sex and of water temperature with THC/PG treatment (Table 7). The post-hoc test confirmed that latency was significantly longer after THC inhalation, compared with vehicle inhalation, for female rats when evaluated at 48ºC and 50ºC. Significantly longer latencies were also confirmed for male versus female rats when tails were placed in 48ºC water after vehicle inhalation. Follow-up two-way ANOVA were conducted within sex groups to further parse these effects. The marginal mean post-hoc analysis confirmed that withdrawal latency was significantly different between vehicle and THC inhalation, and between all three water bath temperatures, for each sex. Since the inhalation conditions were counter-balanced for the main study without respect to estrous stage, not all phases were initially captured for all female rats. Thus, additional sessions were conducted to complete estrus (PG N=4; THC N=5) and diestrus (PG N=6; THC N=5) evaluations in the 50 ºC water-bath so that the full sample (N=8) was available for analysis. In this case the tail withdrawal latency was significantly slowed by THC inhalation in both estrus and diestrus, but this was not different across estrous stage.

Experiment 8: Pharmacokinetics

Analysis of the single time-point blood draws obtained after inhalation of three THC concentrations (25, 100, 200 mg/mL) showed plasma THC varied by concentration in the vehicle but not by sex (Figure 11A). The analyses confirmed a significant effect of Concentration on plasma THC for the 35 minute [$F (2, 30) = 23.18$; $P<0.0001$] and 60 minute samples [$F (2, 34) = 19.79$; $P<0.0001$], but there was no significant effect of Sex or of the interaction of factors. The post-hoc tests also confirmed that for each time point,

higher plasma THC was observed after inhalation of 200 mg/mL compared with either lower concentration in each sex. Analysis of the plasma sampled serially after a single session (30 minutes; THC 100 mg/mL) in the group of five rats (3F) with patent catheters confirmed a significant effect of Time [$F(3, 14) = 11.4$; $P<0.001$] and the post-hoc test confirmed that plasma THC was higher immediately post-inhalation compared with all subsequent time points (Figure 11B). Finally, analysis of plasma sampled from rats after i.p. injection confirmed a significant effect of dose [$F(1, 10) = 5.92$; $P<0.05$], but there were no significant effects of sex or the interaction of factors (Figure 11C).

Discussion:

This study shows that similar effects are observed in male and female rats after inhalation of Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD) and their combination using a recently described (Nguyen et al. 2016b) e-cigarette based vapor technology. The data confirm a similar degree of hypothermia, hypoactivity and antinociception are produced in each sex and that none of these effects differed across estrous and diestrous phases of the estrous cycle in female rats. Similar plasma THC levels were produced in male and female rats under identical inhalation conditions, regardless of a significant difference in body weight, reinforcing the behavioral and thermoregulatory similarity across sexes. Plasma THC concentrations in human vary depending on the potency of marijuana, the manner in which the drug is smoked and individual idiosyncrasies but peak THC plasma concentrations of 62 vs.162 ng/mL have been reported using ad libitum and paced inhalation procedures, respectively (Hartman et al. 2015; Huestis et al. 1992). The present work confirms plasma levels of ~40 ng/mL after 30 minutes inhalation of 100 mg/mL THC and 137 ng/mL after inhalation of 200 mg/mL. Additional studies found an interactive effect when THC and CBD were inhaled simultaneously at threshold concentrations (Figure 7). CBD decreased body temperature in both male and female rats and in a dose-dependent (duration of inhalation of CBD 100 mg/mL; 100 vs 400 mg/mL for 30 min) manner although the CBD effect was less incremental across dose in females and of much smaller maximum extent compared with the effects of THC in both sexes. The additive hypothermic effects of THC and CBD were observed in males and females (and hypolocomotor effects in males) when administered at a 1:4 THC:CBD ratio (at threshold doses of each) but no interactive effects were confirmed for a 2:1 THC:CBD ratio in male rats. Finally, there was no evidence of plasticity in the response to inhaled THC given the dosing schedule, since the magnitude of hypothermia produced by 100 mg/mL did not differ from the first experiment to the final combination experiment (Figure 9).

Although our prior study (Nguyen et al. 2016b) found a significant sex difference in the effects of THC inhalation, this study did not confirm that prior, limited result. It may be that a series of i.v. THC challenges completed in both groups prior to the vapor inhalation led to differential plasticity of the hypothermia response, although prior work suggests female rats develop greater tolerance than males even at a lower mg/kg injected dose (Wakley et al. 2014b). Alternately it may be the case that the limitation of the prior female group to N=5 resulted in an effect of individual differences by chance. In the present study, the THC-induced hypothermia did appear to last slightly longer in the female rats (Figures 2, 9) which may represent increased female rat sensitivity.

The lack of a sex difference in hypothermia in these results may only appear to contrast with some prior results because of the wide dose ranges used. For example, hypothermia was greater in magnitude in female versus male rats after i.p. administration of THC at 100-176 mg/kg, but was equivalent from 1-30 mg/kg (Wiley et al. 2007). Plasma THC levels equivalent to those found in human smokers were produced by 10 mg/kg, i.p. in the present study (Figure 11C) suggesting anything above 30 mg/kg, i.p. in a rat may be a poor match for the human condition. As a caveat, one early study found female Wistar rats developed greater hypothermia than males after lower doses of THC (5-20 mg/kg, i.p.) however the ages were much younger than in the present study (the males in particular had weights consistent with only about one week post-adolescent) and rats were restrained for temperature monitoring (Borgen et al. 1973). Since CB_1 receptor densities in the hypothalamus (associated with thermoregulation) vary across the estrous cycle in female rats (Rodriguez de Fonseca et al. 1994) it may be the case that sex differences would be obscured if estrous stage is not taken into account. However in the present study the magnitude of THC-induced hypothermia, did not vary substantially between estrus and diestrus (Figures 3, 4). The possibility of faster recovery of temperature and activity during estrus observed in the higher-dose (50 mg/mL) experiment was not confirmed in the lower-dose (25 mg/mL) experiment, despite a slower initial development of the response and a quicker resolution compared with the 50 mg/mL THC inhalation.

Acute suppression of locomotor activity following either THC or CBD vapor inhalation in this study was of approximately similar magnitude across both sexes, similar to a prior report of no sex differences in the locomotor effects of 1-176 mg/kg, THC, i.p. (Wiley et al. 2007). This lack of a sex difference was, however, discordant with another study showing that 30 mg/kg THC, *s.c.*, decreased locomotor activity only in male rats (Marusich et al. 2014) as well as with other findings that female rodents are *more* sensitive to the acute locomotor effects of cannabinoids compared with males. These latter differences occurred across all stages of the estrous cycle of the females, suggesting that hormonal levels were not the primary mediators (Tseng and Craft 2001; Tseng et al. 2004). Ovarian hormones do not modulate THC-induced locomotor suppression (Wakley et al. 2014a) and adolescents of both sexes showed comparable locomotor effects following a full agonist cannabinoid (Romero et al. 2002). In accordance with those studies, the present study showed no estrous cycle-dependent differences in THC effects on locomotion activity in females between estrus and diestrus.

The present study also found that the inhalation of THC produced an anti-nociceptive effect in both female and male rats, with a sex by drug condition interaction confirming a larger effect in females (Figure 9). As one minor caveat, response latencies were longer in males than females and two males out of eight passed the 15 seconds cut-off time in the PG condition at 48ºC; four of eight females and 5 of eight males reached the cutoff in the THC condition at the same temperature. The imposed ceiling on the maximum latency might therefore have under-represented the THC effect on males. Nevertheless, these results are reasonably consistent with previous studies in which cannabinoids were more potent in anti-nociception in female than in male rats (Wakley et al. 2014b). As with the temperature response, the anti-nociceptive effects of THC inhalation were not different across estrous and diestrous stages in this study which is similar to a prior finding after i.c.v. THC (Wakley

and Craft 2011). These results were further supported by another study in which THC-induced tail withdrawal anti-nociception was not altered by the administration of estradiol, progesterone, or the combination of both hormones in gonadally intact rats (Wakley et al. 2014a) and overall, THC -induced thermal anti-nociception does not appear to be sensitive to ovarian hormone modulation (Craft and Leitl 2008; Wakley et al. 2014a).

Cannabidiol has variously been shown to either increase, or to oppose, the behavioral effects of THC on temperature responses in rodents when administered by intraperitoneal injection, as recently reviewed (Boggs et al. 2018). One commentary has asserted that oppositional effects may depend on CBD doses eightfold higher than the THC dose (Zuardi et al. 2012) or on significant offset in the time of administration, however neither conditions appear consistent with the smoking or vaping of cannabis or cannabis extracts. We found that CBD, when administered (i.p.) either simultaneously or as a pretreatment, did not decrease the effects of THC on activity and thermoregulation in rats and instead potentiated the THC effects (Taffe et al. 2015). In a mouse model neither intravenous nor inhaled CBD reduced the effects of THC or inhaled marijuana smoke in the tetrad test, however, higher doses of CBD potentiated the anti-nociceptive effects of a low dose of THC and significantly elevated THC blood and brain levels (Varvel et al. 2006). Similarly, higher doses of CBD (10 or 50 mg/kg, i.p.) exacerbated the effects of low dose THC (1 mg/kg, i.p.) on activity, thermoregulation and spatial memory (Hayakawa et al. 2008). The present study did not find oppositional effects of CBD inhalation on the thermoregulatory or locomotor effects of THC inhalation in male and female rats. There was no impact of CBD administered at a 2:1 THC:CBD ratio in male rats and an *additive* effect on hypothermia when administered at a 1:4 THC:CBD ratio in male or female rats, particularly at threshold (Figure 7) THC inhalation concentrations. Interestingly, the inhalation of CBD by itself significantly reduced body temperature of both male and female rats. This contrasts with our prior finding of no thermoregulatory effect of i.p. injection of CBD in male rats (Taffe et al. 2015) and a report of no hypothermia after i.v. CBD in male mice (Varvel et al. 2006) which suggests there may be significant differences that depend on the route of administration. CBD-containing e-cigarette liquids are already appearing on the retail market (Peace et al. 2016), therefore a rodent model is of significant use to further evaluate *in vivo* impacts of inhaled CBD.

Male and female rats exhibited the same degree of maximum hypothermia and the same plasma THC levels after vapor inhalation despite a substantial difference in body size. In particular, the comparison of the effect of identical dosing conditions (100 mg/mL THC) at two different time points across the study (Figure 9) shows similar magnitude of initial hypothermia across sex and across time (and therefore body weight). While total ventilation (Doperalski et al. 2008) and cortical blood flow (Roof and Hall 2000) of age-matched male and female rats are not different, female blood volume (Probst et al. 2006) and brain weight (Bishop and Wahlsten 1999) are lower than that of males. Therefore it might have been predicted that, if anything, females would have experienced a higher dose. No major sex differences were observed across inhalation conditions, thus it is most parsimonious to conclude that any minor sex-dependent differences in effective brain concentrations that may have been reached at a given dosing condition were not large enough to compromise the overall conclusion of minimal sex differences.

In conclusion, this model produces repeatable THC exposure in either rat sex that is both congruent with human THC use, in terms of plasma levels, and congruent with behavioral signs of THC following parenteral injection in rodents. It may be the case that this new model of e-cigarette type vapor inhalation provides an improved technique for the evaluation of cannabinoid effects in rodent pre-clinical models, possibly because the *in vivo* effects have relatively rapid onset and offset relative to injected doses that produce similar magnitudes of effect (Nguyen et al. 2016b; Taffe et al. 2015).

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Week 1	Week 2		Weeks 3-8	Weeks 9-12	Weeks 17-21	Weeks 22-26	Weeks 27-29	Weeks 30-35
Arrival in Lab	*Telemetry Implantation*	Female	Expt. 1	Expt. 2	Expt. 4	Expt. 5	Expt. 6	Expt. 7
		Male	Expt. 1	Expt. 3	Expt. 4	Expt. 5	Expt. 6	Expt. 7

Figure 1:

The timeline indicates the intervals during which each of the telemetry experiments was conducted in the groups of male and female rats.

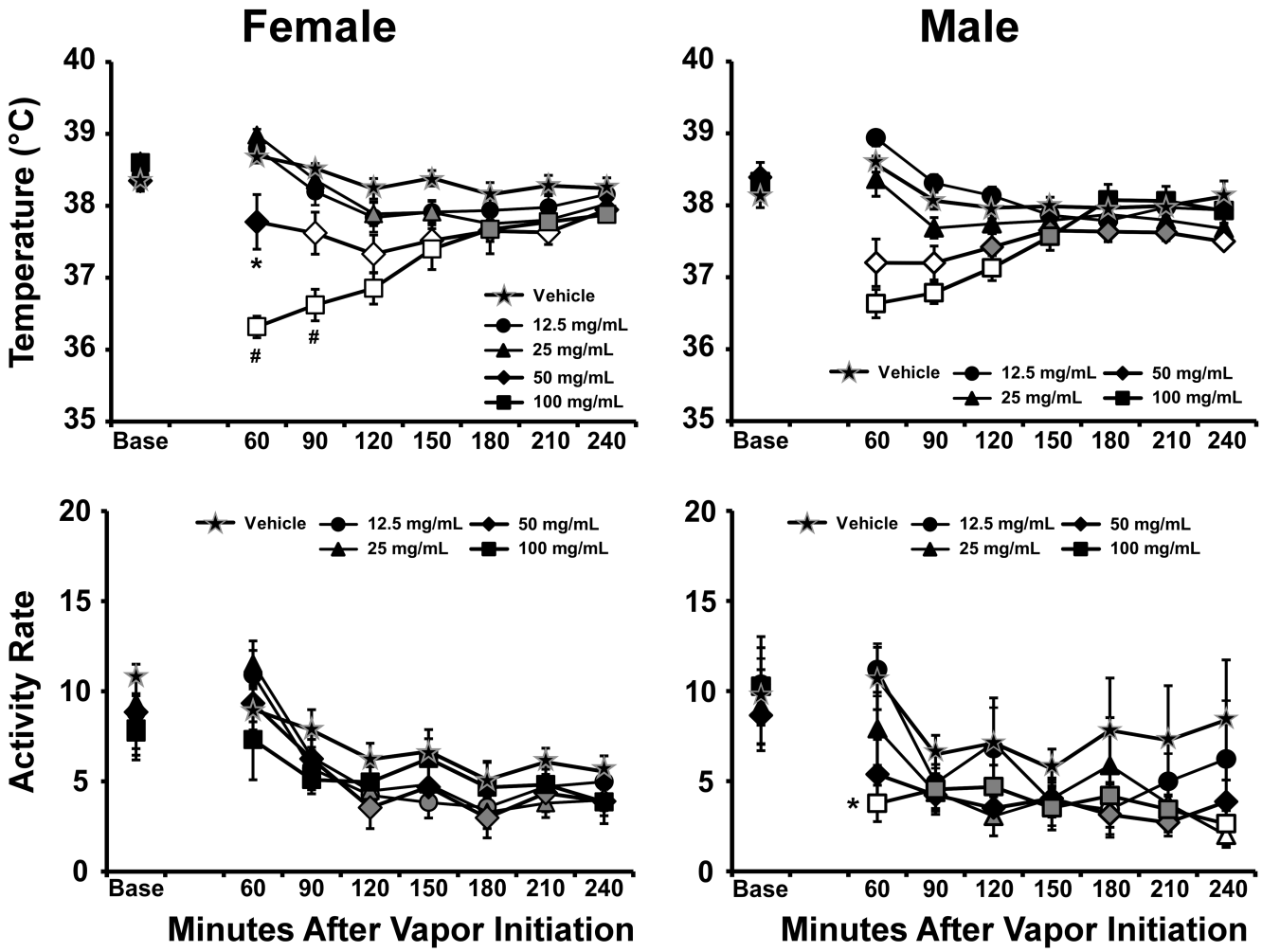

Figure 2:

Mean female (N=8; ±SEM) and male (N=8; ±SEM) body temperatures and activity following inhalation exposure to the polyethylene glycol vehicle (PG) or THC (12.5-100 mg/mL) vapor for 30 minutes. Open symbols indicate a significant difference from both vehicle at a given time-point and the within-treatment baseline, while shaded symbols indicate a significant difference from the baseline only. A significant difference from the vehicle condition (only) at a given time is indicated by * and from the 50 mg/mL condition by #. Base=baseline value.

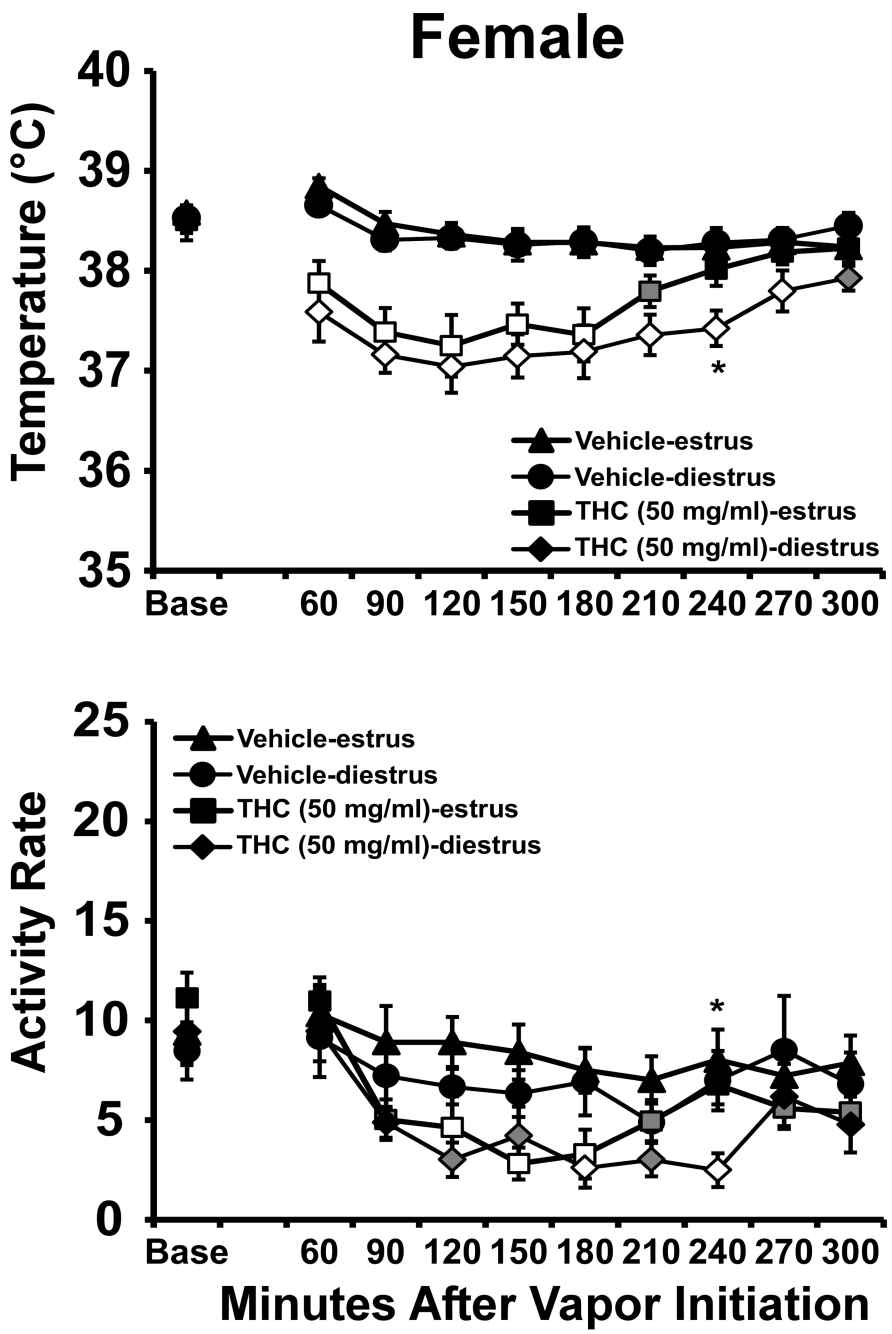

Figure 3:

Mean (N=8; ±SEM) temperature and activity following vapor inhalation of the vehicle or THC (50 mg/mL) for 30 minutes in estrus and diestrus. Open symbols indicate a significant difference from both vehicle and the baseline, while shaded symbols indicate a significant difference from the baseline only; A significant difference between estrus and diestrus in THC 50 mg/mL at a given time is indicated by *

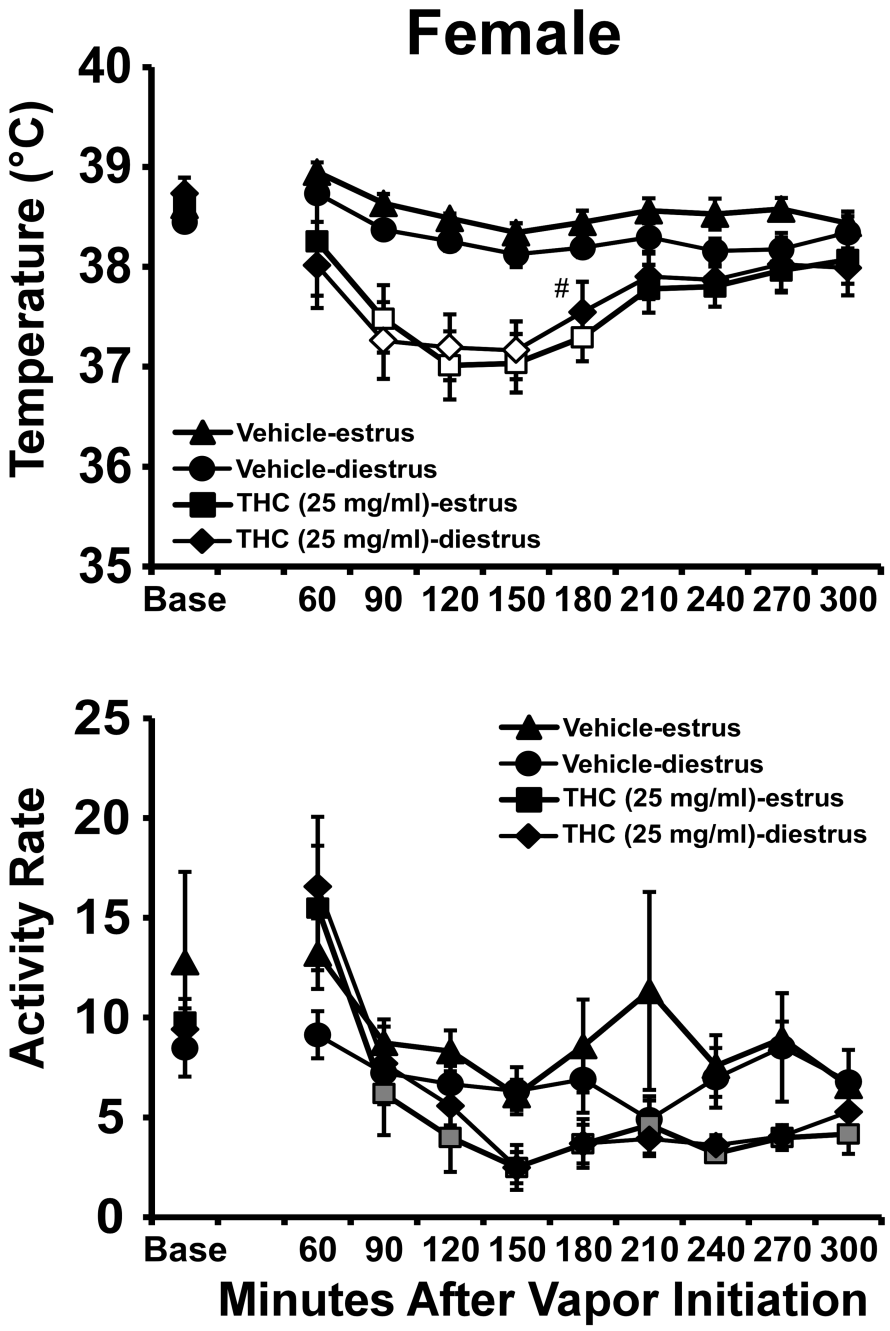

Figure 4:

Mean (N=6-7; ±SEM) temperature and activity of female rats following vapor inhalation of the vehicle or THC (25 mg/mL) for 40 minutes in estrus and diestrus. Open symbols indicate a significant difference from both vehicle and the baseline, while shaded symbols indicate a significant difference from the baseline only; A significant difference from the vehicle condition at a given time and estrous phase is indicated by #.

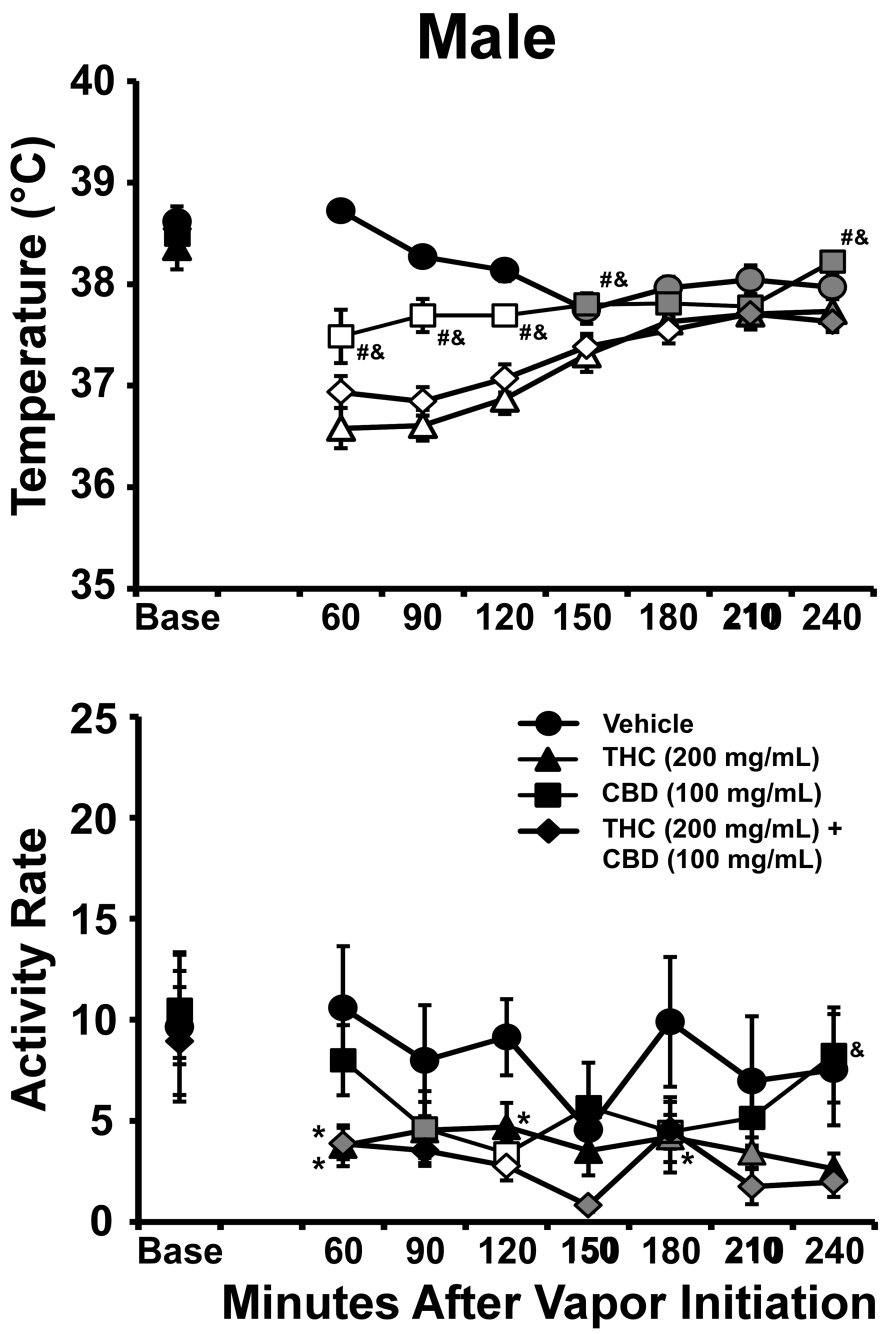

Figure 5:

Mean (N=8; ±SEM) temperature and activity of male rats following vapor inhalation of the vehicle, THC (200 mg/mL in PG), cannabidiol (CBD; 100 mg/mL) or the THC + CBD combination. Open symbols indicate a significant difference from both vehicle (at a given time-point) and the within-treatment baseline, while shaded symbols indicate a significant difference from the baseline only. A significant difference from vehicle condition (only) is indicated by *, from the THC (200 mg/mL) condition at a given time by # and from the THC (200 mg/mL)+CBD (100 mg/mL) condition by &. Base=baseline value.

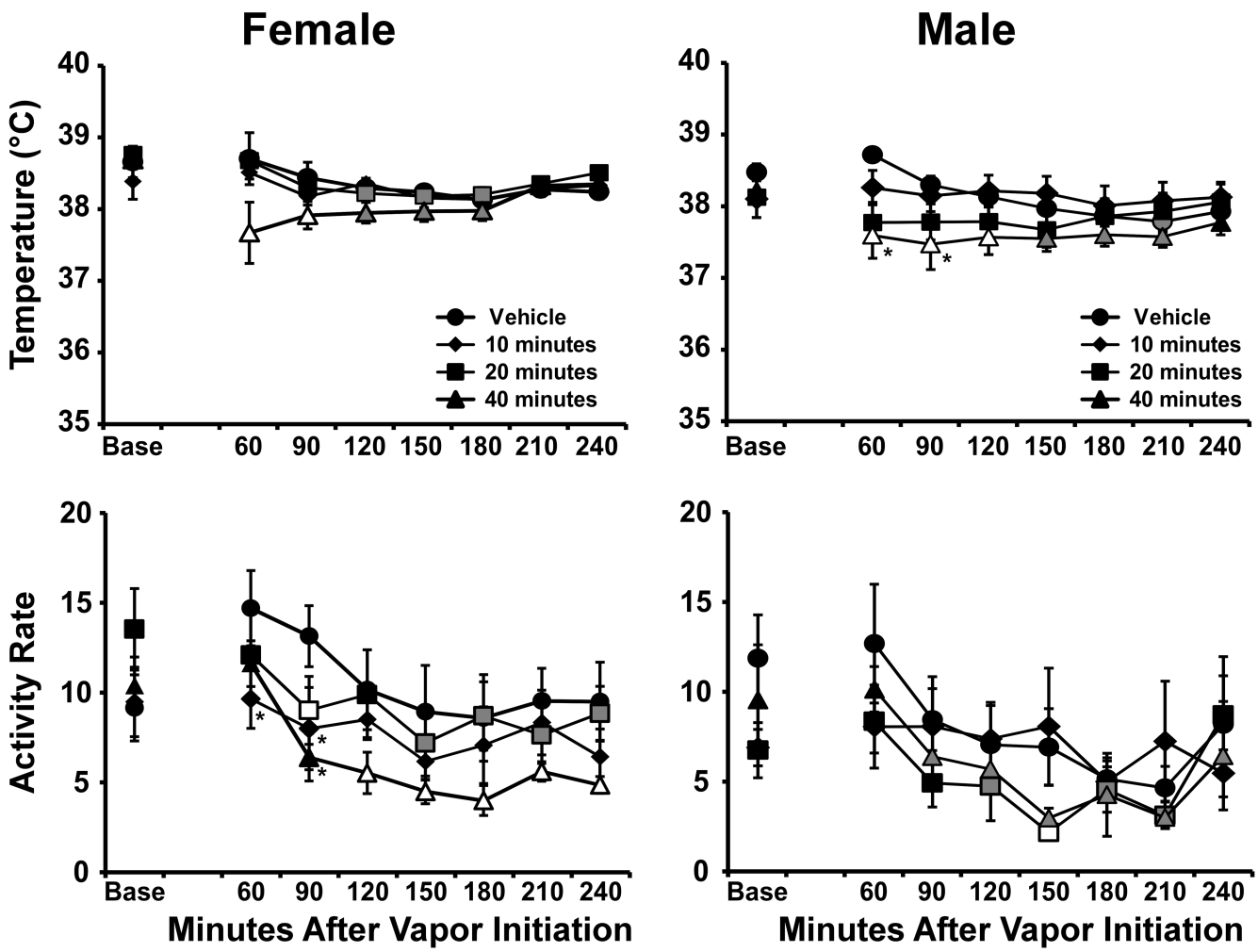

Figure 6:

Mean female (N=8; ±SEM) and male (N=8; ±SEM) body temperatures and activity following inhalation exposure to the vehicle or CBD (100 mg/mL) vapor for 10, 20 and 40 minutes vapor inhalation. Open symbols indicate a significant difference from both vehicle and the baseline, while shaded symbols indicate a significant difference from the baseline only. A significant difference from the vehicle condition (only) at a given time is indicated by *.

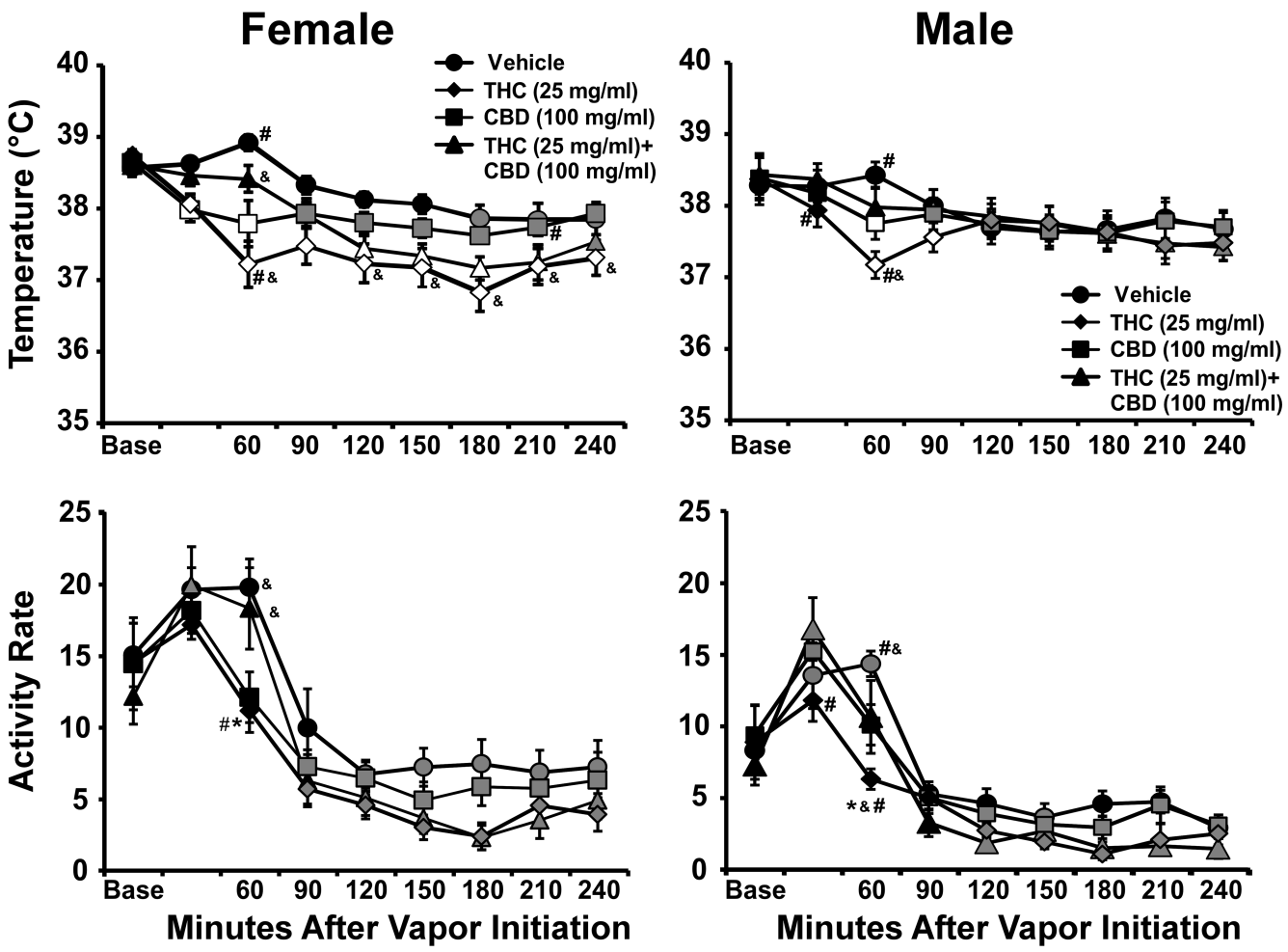

Figure 7:

Mean female (N=8; ±SEM) and male (N=8; ±SEM) temperature and activity following vapor inhalation of the vehicle, THC (25 mg/mL), cannabidiol (CBD; 100 mg/mL) or the THC/CBD combination. Open symbols indicate a significant difference from both vehicle and the baseline, while shaded symbols indicate a significant difference from the baseline only. A significant difference from the vehicle condition (only) at a given time is indicated by *, a difference from THC (25 mg/mL) by # and a significant difference from CBD (100 mg/mL) by &.

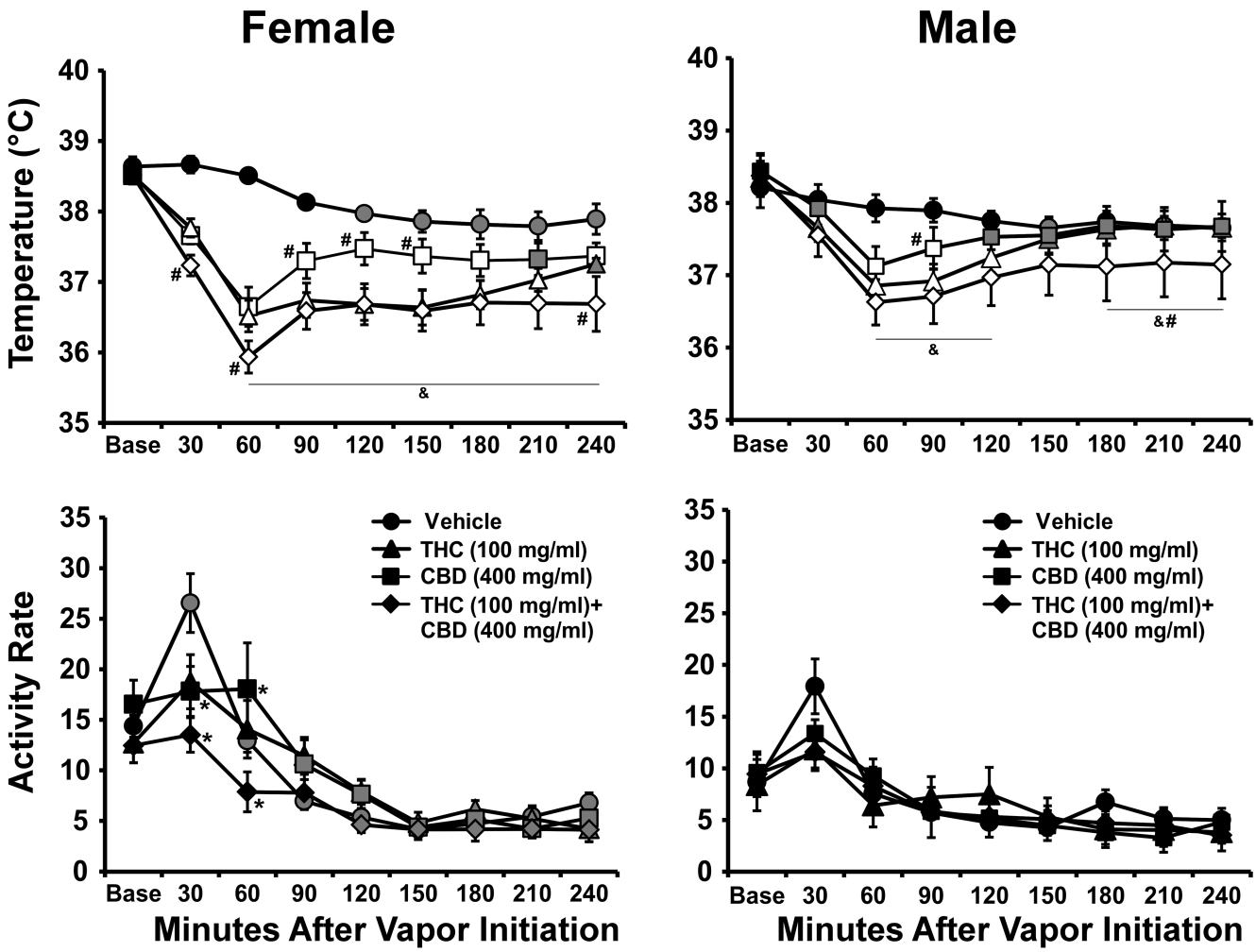

Figure 8:

Mean female (N=8; ±SEM) and male (N=8; ±SEM) temperature and activity following vapor inhalation of the vehicle, THC (100 mg/mL), cannabidiol (CBD; 400 mg/mL) or the THC/CBD combination. Open symbols indicate a significant difference from both vehicle and the baseline while shaded symbols indicate a significant difference from the baseline only. A significant difference from the vehicle condition (only) at a given time is indicated by *, a difference from THC (100 mg/mL) by # and a significant difference from CBD (400 mg/mL) by &.

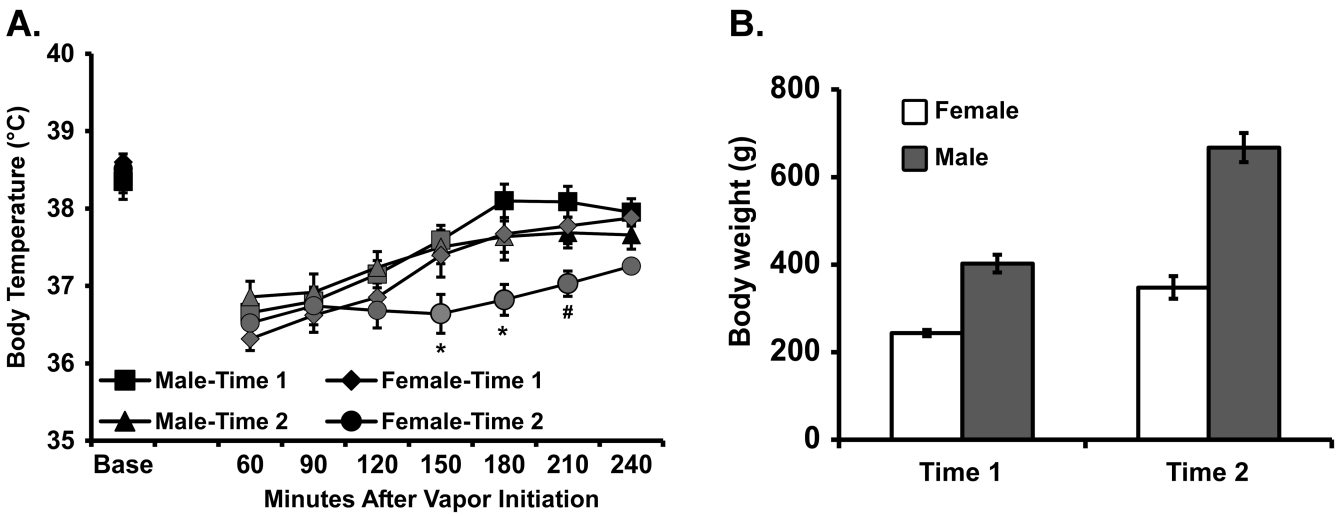

Figure 9:

A) *Mean male and female (N=8 per group; ±SEM) temperature following vapor inhalation of THC (100 mg/mL) for 30 minutes in Experiments 1 (Time 1) and 7 (Time 2). Shaded symbols indicate a significant difference from the baseline. A significant difference from all other groups/Times is indicated by * and from the males at Time 1 by #. B) Mean body weight for male and female groups at Time 1 and Time 2.*

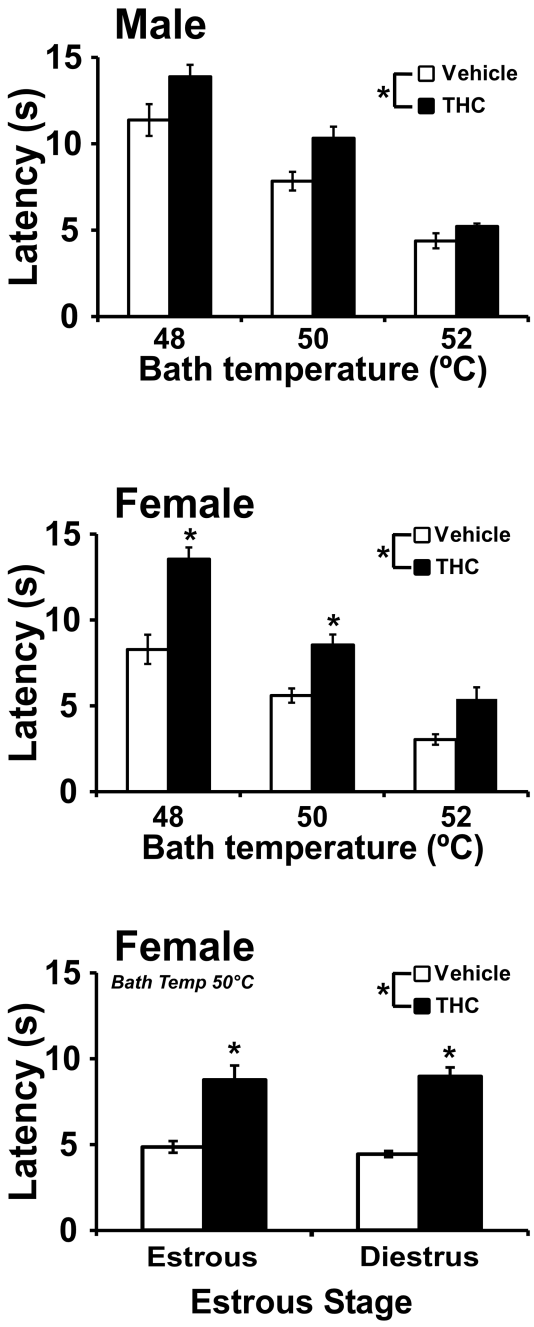

Figure 10:

Mean (N=8; ±SEM) tail withdrawal latency *following vapor inhalation of the vehicle or THC (100 mg/mL) for 30 minutes. Latency was assessed at 35 minutes after the start of inhalation. A significant difference between the vehicle and THC conditions is indicated by **.*

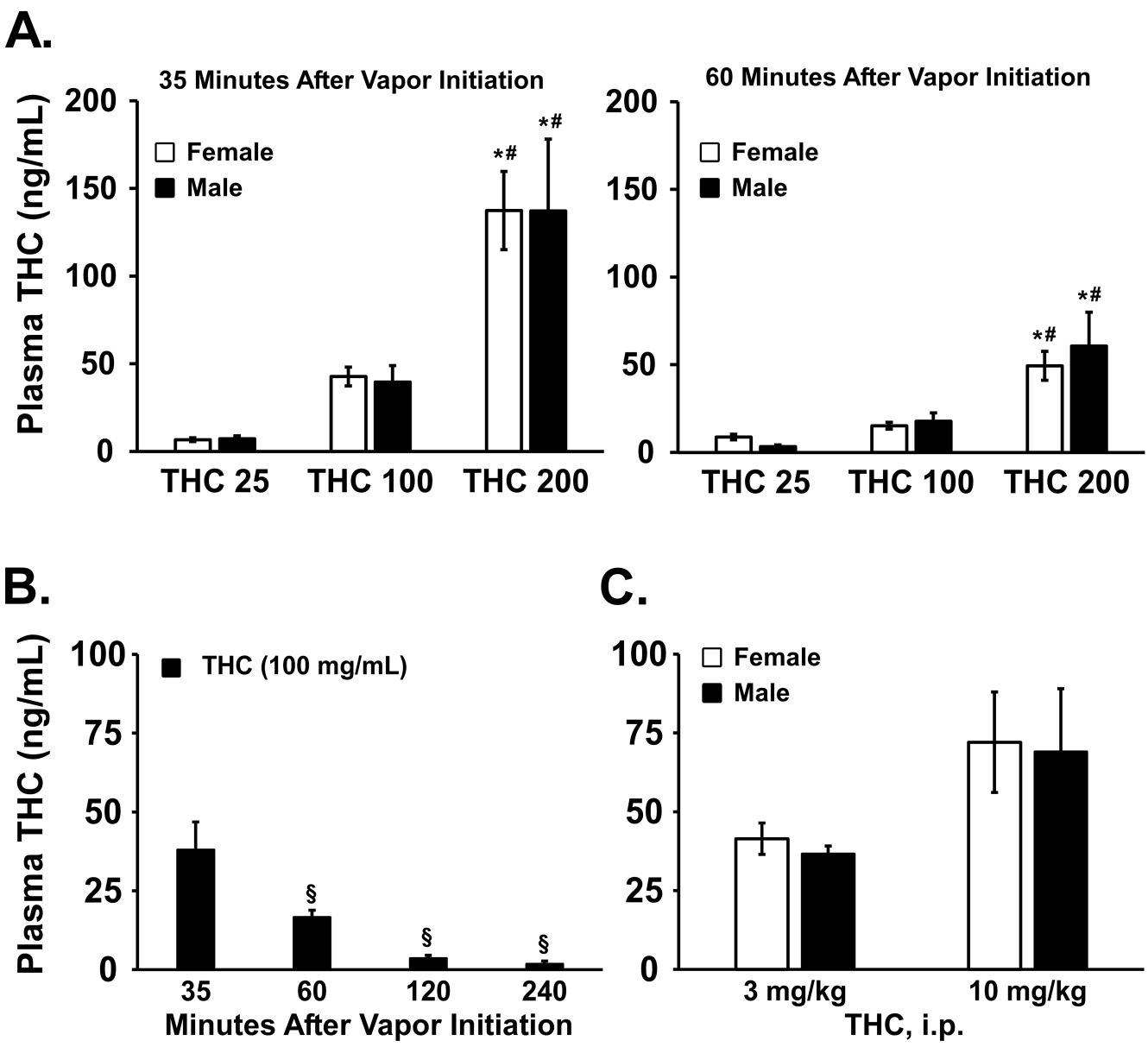

Figure 11:

A) Mean plasma THC levels for male (N= 6 per observation; ±SEM) and female (N= 5-7 per observation; ±SEM) rats following separate sessions of vapor inhalation of the THC (25, 100 or 200 mg/mL) for 30 minutes. A significant difference from the 25 mg/mL condition is indicated by * and from the 100 mg/mL condition by #. B) Mean (N= 5, 3F; ±SEM) plasma THC levels after a single session of vapor inhalation of THC (100 mg/mL) for 30 minutes. C) Mean plasma THC levels for male (N= 3 per observation; ±SEM) and female (N= 4 per observation; ±SEM) after a single injection of THC (3, 10 mg/kg, ip). A significant difference from the 35 minute time point is indicated by §.

Table 1:

The significant ANOVA outcomes for the main factors and interaction of factors are summarized for Experiment 1.

	Sex	Time Post-initiation	Vapor inhalation	Interaction of factors
Body temperature	Female	F(7, 49) = 18.49; P < 0.0001	F(4, 28) = 13.44; P < 0.0001	F(28, 196) = 9.10; P < 0.0001
	Male	F(7, 49) = 8.65; P < 0.0001	F(4, 28) = 11.09; P < 0.0001	F(28, 196) = 6.45; P < 0.0001
Activity	Female	F(7, 49) = 26.78; P < 0.0001	-	-
	Male	F(7, 49) = 16.53; P < 0.0001	F(4, 28) = 3.48; P <0.05	-

Table 2:

The significant ANOVA outcomes for the main factors and interaction of factors are summarized for Experiment 2.

	THC condition	Time Post-initiation	Drug / estrous condition	Interaction of factors
Body temperature	50 mg/mL (30 min)	F(9, 63) = 13.12; P < 0.0001	F(3, 21) = 19.15; P < 0.0001	F(27, 189) =3.42; P < 0.0001
	25 mg/mL (40 min)	F(9, 198) = 14.94; P <0.0001	F(3, 22) = 5.66; P < 0.01	F(27, 198) = 2.83; P < 0.0001
Activity	50 mg/mL (30 min)	F(9, 63) = 13.80, P<0.0001	F(3, 21) = 5.00; P<0.01	-
	25 mg/mL (40 min)	F(9, 198) = 14.52; P < 0.0001	-	-

Table 3:

The significant ANOVA outcomes for the main factors and interaction of factors are summarized for Experiment 3.

	Sex	Time Post-initiation	Vapor inhalation	Interaction of factors
Body temperature	Male	F(7, 49) = 40.83; P<0.0001	F(3, 21) = 37.3; P<0.0001	F(21, 147) = 11.88; P<0.0001
Activity	Male	F(7, 49) = 5.47; P=0.0001	F(3, 21) = 6.06; P<0.005	F(21, 147) = 2.12; P<0.01

Table 4:

The significant ANOVA outcomes for the main factors and interaction of factors are summarized for Experiment 4.

	Sex	Time Post-initiation	Vapor inhalation	Interaction of factors
Body temperature	Female	F(7, 49) = 5.84, P<0.0001	-	F(21, 147) = 3.05; P<0.0001
	Male	F(7, 49) = 3.52; P<0.005	-	F(21, 147) = 2.24; P<0.005
Activity	Female	F(7, 49) = 19.39, P<0.0001	-	F(21, 147) = 1.90; P<0.05
	Male	F(7, 49) = 11.59; P<0.0001	F(3, 21) = 4.63; P<0.05	-

Table 5:

The significant ANOVA outcomes for the main factors and interaction of factors are summarized for Experiment 5.

	Sex	Time Post-initiation	Vapor inhalation	Interaction of factors
Body temperature	Female	F(8, 56) = 22.53; P<0.0001	F(3, 21) = 5.19; P<0.01	F(24, 168) = 4.23; P<0.0001
	Male	F(8, 56) = 15.16; P<0.0001	-	F(24, 168) = 3.36; P<0.0001
Activity	Female	F(8, 56) = 70.27; P=0.0001	-	-
	Male	F(8, 56) = 31.10; P=0.0001	F(3, 21) = 4.97; P<0.01	F(24, 168) = 2.02; P<0.01

Table 6:

The significant ANOVA outcomes for the main factors and interaction of factors are summarized for Experiment 7.

Experiment 7	Sex	Time Post-initiation	Vapor inhalation	Interaction of factors
Body temperature	Female	F(8, 56) = 24.01; P<0.0001	F(3, 21) = 15.3; P<0.0001	F(24, 168) = 5.19; P<0.0001
	Male	F(8, 56) = 12.25; P<0.0001	-	F(24, 168) = 3.64; P<0.0001
Activity	Female	F(8, 56) = 34.82; P<0.0001	-	F(24, 168) = 3.26; P<0.0001
	Male	F(8, 56) = 13.86; P<0.0001	-	-

Table 7:

The significant ANOVA outcomes for the main factors and interaction of factors are summarized for Experiment 6.

	Sex	**Vapor inhalation**	**Water bath temperature**	**Interaction of THC treatment condition and sex**	**Interaction of water temperature with Vapor treatment**	**Within sex interaction of Water Temperature**		**Within sex interaction of Vapor condition**	
within sex interaction	F(1, 2) = 16.3; P < 0.0001	F(1, 2) = 59.8; P < 0.0001	F(2, 2) = 140; P < 0.0001	F(1, 2) = 5.01; P < 0.05	F(2, 2) = 3.48; P<0.05	Female	Male	Female	Male
						F(2, 14) = 106.9; P<0.0001	F(2, 14) = 168.6; P<0.0001	F(1, 7) = 65.27; P<0.0001	F (1, 7) = 12.87; P<0.01
within estrous phase interaction	NA	F(1, 7) = 51.51; P < 0.001	NA	NA	NA	NA	NA	NA	