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

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Running Head: THC inhalation in male and female rats

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Abstract

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. The aim of this study was 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.

Groups of male and female Sprague-Dawley rats (N=8 per group) were implanted with radiotelemetry devices for the assessment of body temperature and locomotor activity. Animals were then exposed to THC or CBD vapor (4 puffs/5 minutes) using a propylene glycol (PG) vehicle. THC dose was adjusted via the concentration in the vehicle (12.5-200 mg/ml) and 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.

THC inhalation reduced body temperature and increased tail-withdrawal latency in both sexes equivalently and in a concentration-dependent manner. Female temperature, activity and anti-nociceptive responses to THC did not differ between the estrus and diestrus phases. CBD inhalation alone induced 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.

In summary the inhalation of THC or CBD, alone and in combination, produces approximately equivalent effects in male and female rats. Sex differences were subtle and mostly reflected in a more prolonged body temperature disruption in females.

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

Introduction

Human ingestion of *Cannabis sativa* is presumably reinforced by the effects of the phytocannabinoid Δ^9-tetrahydrocannabinol (THC) on the central nervous system. THC is a partial agonist at the endogenous cannabinoid receptors (CB_1 and CB_2) and is the major psychoactive component of most recreational cannabis (Burgdorf et al. 2011; ElSohly et al. 2016; Morgan et al. 2010). 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 (Craft et al. 2012; Romero et al. 2002; 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 sex differences in subjective intoxication or plasma levels of THC were found in humans after smoking marijuana (Mathew et al. 2003; Miller et al. 1983; Wall et al. 1983) and another study found no sex differences in the effects of THC on impulsivity in human subjects (McDonald et al. 2003). In laboratory findings, male rodents are more sensitive than females to the hyperphagic effect of cannabinoid agonists (Diaz et al. 2009) and females are 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). The fact that cannabinoids bind with greater affinity to CB_1 receptors in female compared with male rats (Craft et al. 2012), and that CB_1 expression differs between normally cycling females and either male or ovarectomized female rats (Riebe et al. 2010) suggests a mechanism and further indicates that observed sex differences may vary across the estrous cycle. Thus it remains of significant interest to determine similarities and differences in the effects of THC across sexes.

Humans typically smoke cannabis, and more recently are turning to noncombusted inhalation techniques (Jones et al. 2016; Morean et al. 2015; Varlet et al. 2016), yet almost all preclinical sex differences studies 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 1976; Fried and Nieman 1973; Manwell et al. 2014; Naef et al. 2004; Niyuhire et al. 2007; Wilson et al. 2002; Wilson et al. 2006). Parenteral administration of cannabinoid agonists suppresses spontaneous activity, decreases nociception, induces hypothermia, and increases catalepsy in rodents of both sexes (Compton et al. 1993; Martin et al. 1991; 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 levels of antinociception, hypothermia, and catalepsy in mice exposed to the THC aerosol (Lichtman et al. 2000, Wilson, 2002 #57) 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, further confirming that inhalation delivery of THC is a viable method to elicit cannabinoid-typical pharmacological effects (Lichtman et al. 2001).

An additional phytocannabinoid of recent interest, cannabidiol (CBD), does not activate the CB_1 and CB_2 receptors (Pertwee 2006; 2008) but has shown activity as an antagonist 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 cause by THC, i.p. when administered in 1:1-1:3 THC:CBD ratios in rats (Taffe et al. 2015) and only increased the discriminative stimulus effects of THC administered intravenously in monkeys, albeit at very low THC:CBD ratios (McMahon 2016). It remains unknown how CBD may alter the effects of THC when administered by inhalation.

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 and activity. Experiments were included to assess THC effects during estrus and diestrus to determine any possible contributions of the estrous cycle. Finally, the effects of inhaled CBD on thermoregulation and activity were determined in isolation and in combination with THC.

Materials and methods

Subjects: Age matched groups of male (N=8) and female (N=8) Wistar rats (Charles River, NC, USA) were housed in humidity and temperature-controlled (23±1 °C) vivaria on 12:12 hour light:dark cycles. Animals entered the laboratory at ~10 weeks of age. Animals had *ad libitum* access to food and water in their home cages. All procedures were conducted in the animals' dark (active) cycle under protocols approved by the Institutional Care and Use Committees 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: 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. Absorbable sutures were used to close the abdominal muscle

incision and the skin incision was closed with the tissue adhesive. For the first three days of the recovery period, an antibiotic (cephazolin; 0.4 g/ml; 2.0 ml/kg, s.c.) and an analgesic (flunixin; 2.5 mg/ml; 2.0 ml/kg, s.c.) were administered daily. A minimum of 7 days was allowed for surgical recovery prior to starting any experiments. This minimally invasive technique is useful for characterizing increases and decreases in both body temperature and locomotor activity consequent to drug exposure in both monkeys (Crean et al. 2007; Taffe 2011; 2012; Taffe et al. 2006) and rats (Miller et al. 2013a; Miller et al. 2013b).

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 (Aarde et al. 2013; Miller et al. 2013b; Wright et al. 2012).

***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 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; La Jolla Alcohol Research, Inc, La Jolla, CA, USA) was triggered to deliver the scheduled series of puffs by either MedPC IV software (Med Associates, St. Albans, VT USA) or 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 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 any possible tissue damage (Wakley and Craft 2011; Wakley et al. 2014a). Tail withdrawal was assessed 60 minutes after the initiation of inhalation (i.e. 30 minutes after termination of each session).

Drugs: The inhalation exposure was to Δ^9-tetrahydrocannabinol (THC; 12.5, 25, 50, 100, 200 mg/mL) or cannabidiol (100 mg/mL) in propylene glycol (PG) vehicle. 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 4:12 minute 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.

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 the estrus stage. 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 the morning of the experimental treatment day to confirm. The estrus and diestrus 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).

Experiments:

A minimum 7 day interval separated all active THC dosing for a given individual throughout the following experimental conditions. The following experiments are listed in the order conducted but are presented in the results in a slightly different order for narrative clarity.

Experiment 1: 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 30-minute telemetry data were used as the pre-treatment baseline for statistical analysis purposes. Then, rats were transferred in pairs to separate inhalation chambers for vapor exposure and thereafter returned to their recording cages (this approach was used for Experiments 2-4). In this first experiment, telemetry recording was conducted following dosing conditions of PG or THC (12.5, 25, 50, 100 mg/mL) with the order randomized in 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 randomized within experiment as described below.

Experiment 2: After Experiment 1, the female telemetry group was recorded during the estrus and diestrus phases of the estrous cycle. First the female rats were recorded following 30 minutes of inhalation of THC 50 mg/mL (versus PG) in estrus and diestrus phases 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: 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 randomized order.

Experiment 4: 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 randomized order.

Experiment 5: After Experiment 4, both male and female rat groups were recorded 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 recording was from rats in the inhalation chamber. All 8 animals within the male/female groups were run simultaneously (different inhalation conditions) on a given day. Male and female groups were evaluated on different days.

Experiment 6: 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 in randomized order. After this, the female group was evaluated for nociception after inhalation of PG or THC (100 mg/mL) for 30 minutes in the estrus and diestrus phases of the estrous cycle, in randomized order.

Experiment 7: Finally, both male and female rats were recorded following inhalation of PG, CBD (400 mg/ml), THC (100 mg/mL) or the combination for 30 minutes in randomized 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.

Data Analysis

The body 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 minutes samples). Any missing temperature values were interpolated from preceding and succeeding values. Telemetry data were analyzed with Analysis of Variance (ANOVA) with repeated measures factors for the Drug Treatment Condition, the Time post-

initiation of vapor and estrus phase where relevant. Tail-flick latencies were analyzed with ANOVA with repeated measures factors of Drug Treatment Condition and Water Bath temperature and between-groups factor of sex. Any significant effects within group were followed with post-hoc analysis using Tukey correction for all comparisons. All analysis used Prism 6 or 7 for Windows (v. 6.07 and 7.00; 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 body *temperature* of female rats was decreased by THC inhalation in a concentration dependent manner (**Figure 1**). The ANOVA confirmed significant effects of Time Post-initiation [$F(7, 49) = 18.49$; $P < 0.0001$], of Vapor Conditions [$F(4, 28) = 13.44$; $P < 0.0001$] and of the interaction of factors [$F(28, 196) = 9.10$; $P < 0.0001$]. Temperature was significantly different from the baseline after inhalation of 25 mg/mL (120-240 minutes), 50 mg/mL (90-210 minutes) or 100 mg/mL (60-240 minutes) THC but not after the 12.5 mg/mL 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 after 50 mg/mL (60-150, 210 minutes) or 100 mg/mL (60-150 minutes) THC inhalation. Temperature was also significantly different 60-90 minutes after the start of inhalation of 50 mg/mL versus 100 mg/mL. In addition, the temperature was significantly lower compared with the 12.5 mg/mL condition following 50 mg/mL (60-90 minutes) or 100 mg/mL (60-120 minutes) and lower than the 25 mg/mL following 50 mg/mL (60-120 minutes) or 100 mg/mL (60-120 minutes).

The *activity* of female rats was significantly affected by Time Post-initiation [$F(7, 49) = 26.78$; $P < 0.0001$] but not by Vapor Condition or by the interaction of factors. The marginal mean post-hoc analysis of the Time factor confirmed significantly lower activity rates compared either with the baseline (90-240 minutes post-initiation) or with the 60 minute time-point (90-240 minutes).

Males: The body *temperature* of male rats was also decreased by THC inhalation (**Figure 1**) and the ANOVA confirmed significant effects of Time Post-initiation [$F(7, 49) = 8.65$; $P < 0.0001$], of the five Vapor Conditions [$F(4, 28) = 11.09$; $P < 0.0001$] and of the interaction of factors [$F(28, 196) = 6.45$; $P < 0.0001$]. The temperature was significantly different from the baseline after 50 mg/mL (60-240 minutes) or 100 mg/mL (60-150 minutes) THC inhalation but not after the 12.5 mg/mL, 25 mg/mL or PG conditions. The post-hoc test confirmed significant differences from vehicle after 50 mg/mL (60-90, 240 minutes) or 100 mg/mL (60-120 minutes) THC inhalation. Temperature did not differ compared with PG

in the 12.5 or 25 mg/mL concentrations at any time post-initiation of vapor. In addition, the temperature of male rats was significantly lower compared with the 12.5 mg/mL condition following 50 mg/mL (60-120 minutes) or 100 mg/mL (60-120 minutes) and lower than the 25 mg/mL following 50 mg/mL (60 minutes) or 100 mg/mL (60-120 minutes).

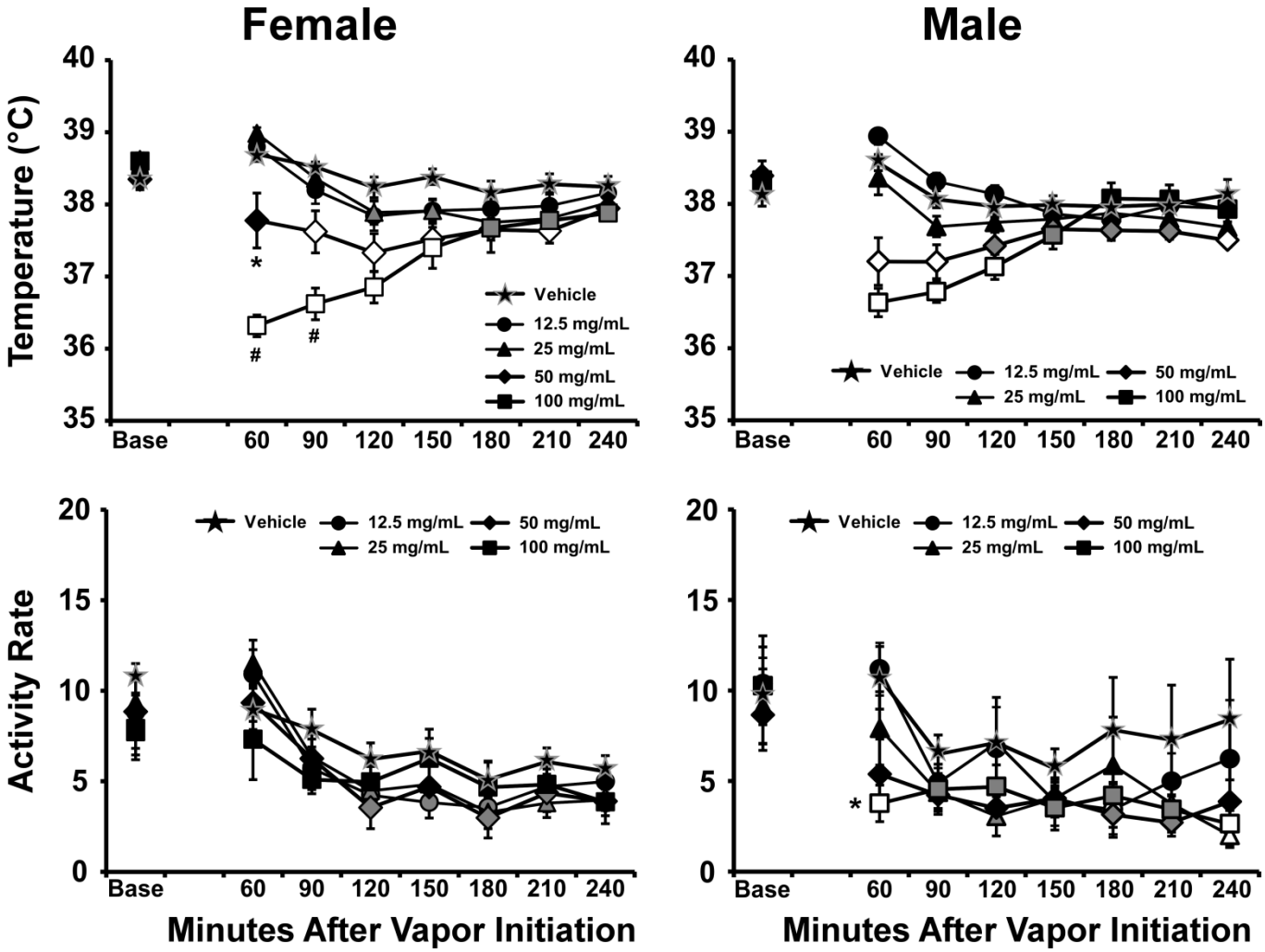

***Figure 1**: Mean female (N=8; ±SEM) and male (N=8; ±SEM) body temperatures and activity following inhalation exposure to the polyethylene glycol vehicle (PG) or THC (12.5-100 mg/mL in PG) vapor for 30 minutes. Open symbols indicate a significant difference from both vehicle at a given time-point and the within-treatment baseline, while shaded symbols indicate a significant difference from the baseline only. A significant difference from the vehicle condition at a given time is indicated by * and from the 50 mg/mL condition by #. Base=baseline value.*

The *activity* of male rats was significantly affected by Time Post-initiation [F (7, 49) = 16.53; P < 0.0001] and by Vapor Condition [F (4, 28) = 3.48; P <0.05] but not by the interaction of factors. 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. Likewise the marginal mean post-hoc analysis of the Time factor confirmed significantly lower activity rates were observed compared with the baseline (90-240 minutes post-initiation) and compared with the 60 minute time-point (90-240 minutes).

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 timepoint [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 3: Vapor Inhalation of THC with Cannabidiol (CBD):

Males: Males were next evaluated in conditions of PG versus CBD (100 mg/mL) for 30 minutes in randomized order and then PG, THC (200 mg/mL) and THC (200 mg/mL) + CBD (100 mg/mL) for 30 minutes in randomized order. Preliminary analysis identified no differences between the first and second PG conditions and thus the second one was used for analysis purposes. Body temperature was again decreased by cannabinoid inhalation (**Figure 2**) and the ANOVA confirmed significant effects of Time Post-initiation [F (7, 49) = 40.83; P<0.0001], of the four Vapor Conditions [F (3, 21) = 37.3; P<0.0001] and of the interaction of factors [F (21, 147) = 11.88; P<0.0001]. The Tukey post-hoc test confirmed that body temperature was significantly different from the baseline after PG (150-240 minutes), CBD 100 mg/mL (60-240 minutes), THC 200 mg/mL (60-240 minutes), and after THC 200 mg/mL + CBD 100 mg/mL (60-240 minutes) inhalation. Correspondingly, body temperature was significantly lower than the PG inhalation condition following CBD 100 mg/mL (60-120 minutes), THC 200 mg/mL (60-150 minutes), and after THC 200 mg/mL + CBD 100 mg/mL (60-180 minutes) inhalation. In addition, significant differences from the CBD 100 mg/mL condition were confirmed for THC 200 mg/mL (60-150, 240 minutes), and after THC 200 mg/mL + CBD 100 mg/mL (60-150, 240 minutes) inhalation. Body temperature was never different between the THC 200 mg/mL and THC 200 mg/mL + CBD 100 mg/mL inhalation conditions.

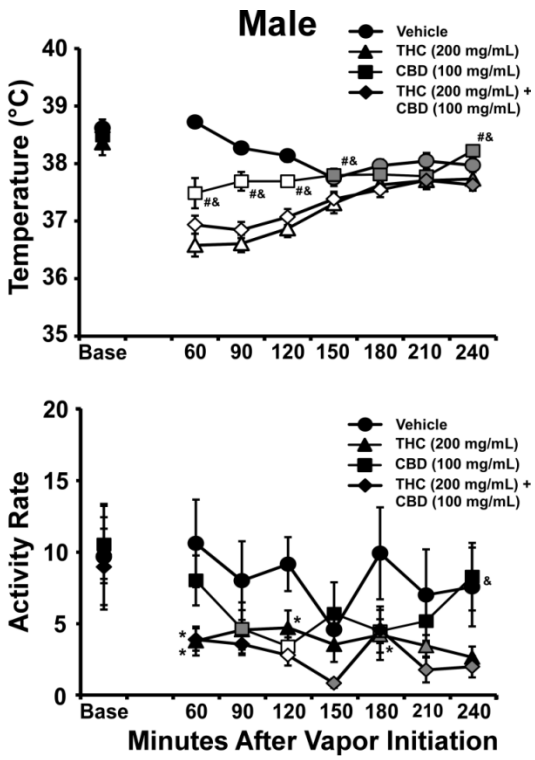

Figure 2: *Mean (N=8; ±SEM) temperature and activity following vapor inhalation of the PG, THC (200 mg/mL in PG), cannabidiol (CBD; 100 mg/mL) or the THC /CBD combination. Open symbols indicate a significant difference from both vehicle at a given time-point and the within-treatment baseline, while shaded symbols indicate a significant difference from the baseline only. A significant difference from vehicle 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.*

Activity rate was also altered by drug inhalation conditions and the ANOVA confirmed significant effects of Time Post-initiation [F (7, 49) = 5.47; P=0.0001], of the four Vapor Conditions [F (3, 21) = 6.06; P<0.005] and of the interaction of factors [F (21, 147) = 2.12; P<0.01]. The Tukey post-hoc test confirmed that activity was significantly different from the baseline after CBD 100 mg/mL (90-120,180 minutes), THC 200 mg/mL (210 minutes), and after THC 200 mg/mL + CBD 100 mg/mL (120-150, 210-240 minutes) inhalation. Activity was significantly lower than the PG inhalation condition following CBD 100 mg/mL (120 minutes), THC 200 mg/mL (60, 120, 180 minutes), and after THC 200 mg/mL + CBD 100 mg/mL (60, 120 minutes) inhalation. A significant difference from the CBD 100 mg/mL condition was confirmed for the THC 200 mg/mL + CBD 100 mg/mL condition 240 minutes after the start of inhalation. 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 body *temperature* of female rats was decreased by CBD (**Figure 3**) and the ANOVA confirmed significant effects of Time Post-initiation [F (7, 49) = 5.84, P<0.0001] and of the interaction of Time Post-initiation with Vapor Condition [F (21, 147) = 3.05; P<0.0001]. The Tukey post-hoc test confirmed that temperature was significantly lower compared with the baseline following inhalation of CBD for 20 minutes (120-180 minutes after the start of inhalation) and for 40 minutes (60-

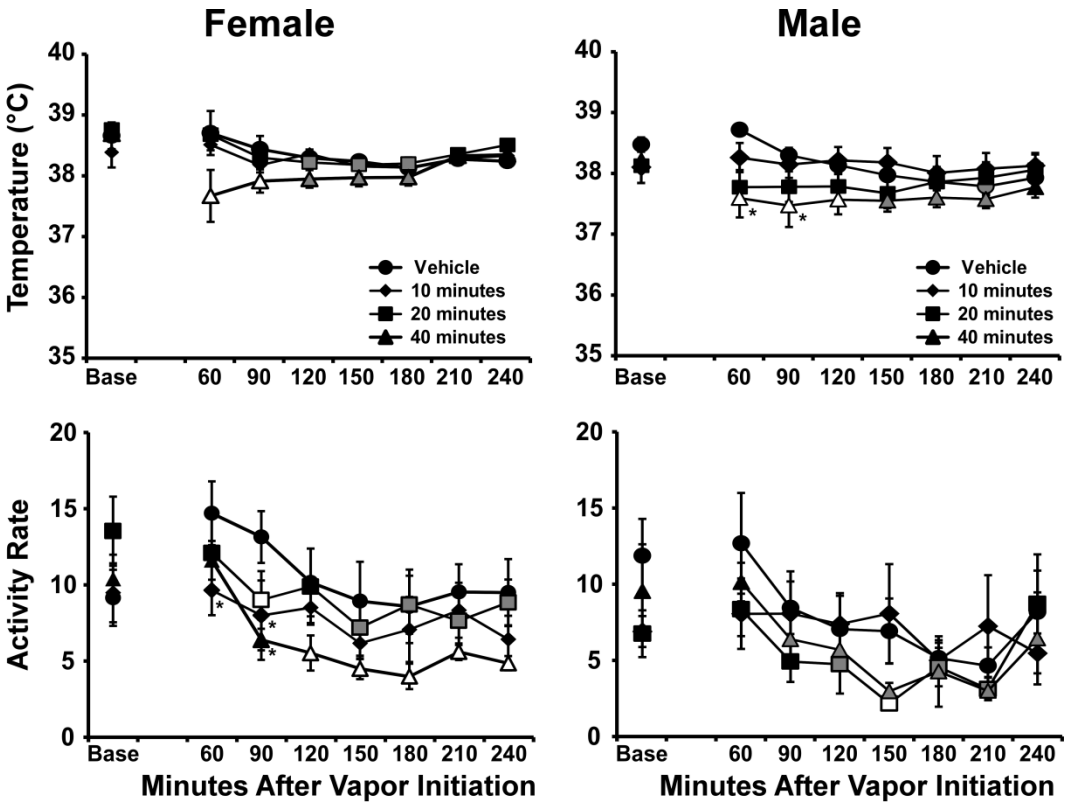

Figure 3: *Mean female (N=8; ±SEM) and male (N=8; ±SEM) body temperatures and activity following inhalation exposure to the polyethylene glycol vehicle (PG) or CBD (100 mg/mL in PG) vapor for 10, 20 and 40 minutes vapor inhalation. Open symbols indicate a significant difference from both vehicle and the baseline, while shaded symbols indicate a significant difference from the baseline only. A significant difference from the vehicle condition at a given time is indicated by *.*

180 minutes). Temperature was also significantly lower compared with the PG condition following inhalation of CBD for 40 minutes (60-90 minutes after the start of inhalation).

The ANOVA also confirmed significant effects of Time Post-initiation [$F(7, 49) = 19.39$, $P<0.0001$] and of the interaction of Time Post-initiation with Vapor Condition [$F(21, 147) = 1.90$; $P<0.05$] on *activity* rate. The Tukey post-hoc test confirmed that activity was significantly lower compared with the baseline following inhalation of CBD for 20 minutes (90, 150-240 minutes after the start of inhalation) or 40 minutes (120-240 minutes after the start of inhalation). Activity rate was also significantly lower compared with the PG condition following inhalation of CBD for 10 minutes (60-90 minutes after the start of inhalation) as well as CBD for 20 minutes (90 minutes); and lower compared with the baseline following inhalation of CBD for 40 minutes (90-240 minutes).

Males: The body *temperature* of male rats (**Figure 3**) was again altered by CBD and the ANOVA confirmed significant effects of Time Post-initiation [$F(7, 49) = 3.52$; $P<0.005$] and of the interaction of Time Post-initiation with Vapor Condition [$F(21, 147) = 2.24$; $P<0.005$]. The Tukey post-hoc test confirmed that temperature was significantly lower compared with the baseline following inhalation of PG (180-210 minutes after the start of inhalation) as well as CBD for 40 minutes (60-210 minutes). Temperature was also significantly lower compared with the PG condition following inhalation of CBD for 20 minutes (60-90 minutes after the start of inhalation) or 40 minutes (60-120 minutes) and lower compared with the CBD 10 minutes condition following inhalation of CBD for 20 minutes (60, 150 minutes after the start of inhalation) or 40 minutes (60-150, 210 minutes).

Male rat *activity* rate was also affected by vapor inhalation and the ANOVA confirmed significant effects of Time Post-initiation [$F(7, 49) = 11.59$; $P<0.0001$] and of Vapor Condition [$F(3, 21) = 4.63$; $P<0.05$], but not of the interaction of factors, on activity rate. The post-hoc of the marginal means confirmed that activity was lower in the 20 minutes CBD inhalation condition compared with PG and across treatment conditions, activity was lower than the baseline (120-210 minutes after the start of inhalation) or the 40 minutes (90-240 minutes) time-points.

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

Females: The body temperature of female rats was decreased by drug inhalation (**Figure 4**) and the ANOVA confirmed significant effects of Time Post-initiation [$F(8, 56) = 22.53$; $P<0.0001$], of Vapor Condition [$F(3, 21) = 5.19$; $P<0.01$] and of the interaction of factors [$F(24, 168) = 4.23$; $P<0.0001$]. The Tukey post hoc test confirmed that body temperature was significantly different from the baseline after PG (180-240 minutes), CBD 100 mg/mL (30-240 minutes), THC 25 mg/mL (90-240 minutes), and after THC 25 mg/mL + CBD 100 mg/mL (30-240 minutes) inhalation. Correspondingly, body temperature was

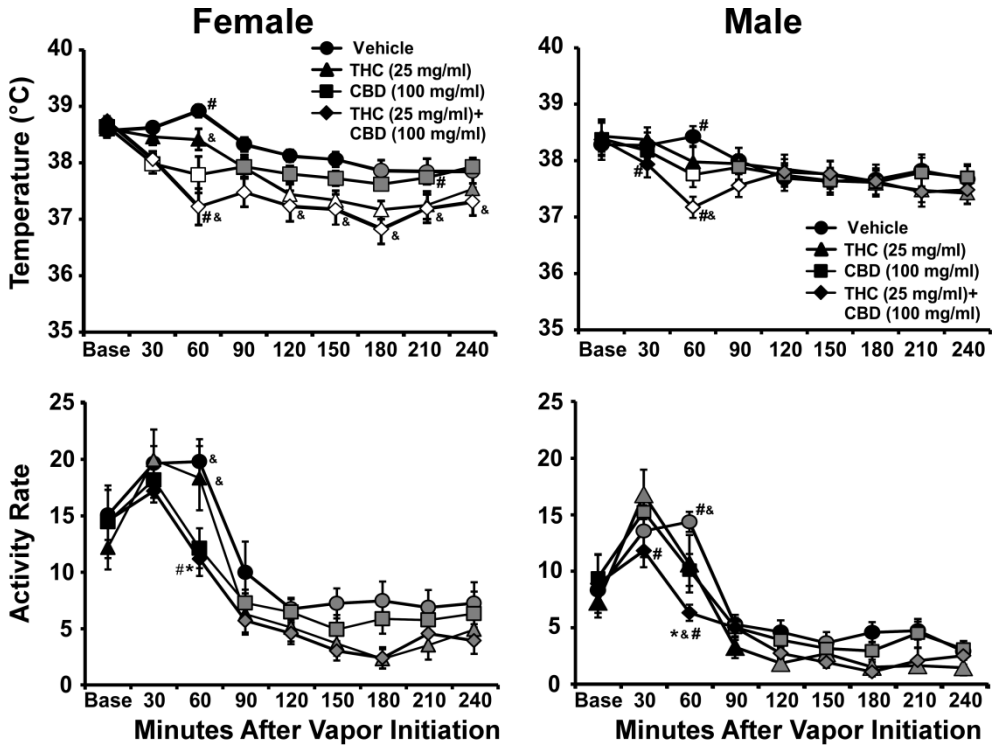

Figure 4: *Mean female (N=8; ±SEM) and male (N=8; ±SEM) temperature and activity following vapor inhalation of the PG, THC (25 mg/mL in PG), cannabidiol (CBD; 100 mg/mL) or the THC/CBD combination. Open symbols indicate a significant difference from both vehicle and the baseline, while shaded symbols indicate a significant difference from the baseline only. A significant difference from the vehicle condition 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 &.*

significantly lower than the PG inhalation condition following CBD 100 mg/mL (30-60 minutes), THC 25 mg/mL (60, 120-210 minutes), and after THC 25 mg/mL + CBD 100 mg/mL (30-240 minutes) inhalation. In addition, significant differences from the CBD 100 mg/mL condition were confirmed for THC 25 mg/mL (60, 210 minutes), and for THC 25 mg/mL + CBD 100 mg/mL (60, 120-240 minutes) inhalation. Body temperature was also different between the THC 25 mg/mL and THC 25 mg/mL + CBD 100 mg/mL, (60 minutes) after inhalation conditions.

The ANOVA confirmed significant effects of Time Post-initiation [F (8, 56) = 70.27; P=0.0001] on activity rate. The Tukey post hoc test confirmed that activity was significantly different from the baseline after PG (120-240 minutes), CBD 100 mg/mL (90-240 minutes), THC 25 mg/mL (30, 120-240 minutes), and after THC 25 mg/mL + CBD 100 mg/mL (90-240 minutes) inhalation. Activity was significantly lower than the PG inhalation condition following CBD 100 mg/mL (60 minutes) and after THC 25 mg/mL + CBD 100 mg/mL (60 minutes) 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, 60 minutes after the start of inhalation.

Males: The ANOVA confirmed significant effects of Time Post-initiation [F (8, 56) = 15.16; P<0.0001], and of the interaction of factors [F (24, 168) = 3.36; P<0.0001] on body temperature (**Figure 4**). The Tukey post hoc test confirmed that body temperature was significantly different from the baseline after PG (120-180, 240 minutes), CBD 100 mg/mL (60-240 minutes), THC 25 mg/mL (90-240 minutes), and

after THC 25 mg/mL + CBD 100 mg/mL (60-240 minutes) inhalation. Correspondingly, body temperature was significantly lower than the PG inhalation condition following CBD 100 mg/mL (60 minutes), THC 25 mg/mL (60 minutes), and after THC 25 mg/mL + CBD 100 mg/mL (60-90 minutes) inhalation. In addition, significant differences from the CBD 100 mg/mL condition were confirmed for THC 25 mg/mL + CBD 100 mg/mL (60 minutes) inhalation. Body temperature was also different between the THC 25 mg/mL and THC 25 mg/mL + CBD 100 mg/mL (30-60 minutes) conditions.

The ANOVA confirmed significant effects of Time Post-initiation [$F(8, 56) = 31.10$; $P=0.0001$], of Vapor Condition [$F(3, 21) = 4.97$; $P<0.01$] and of the interaction of factors [$F(24, 168) = 2.02$; $P<0.01$] on activity rate. The Tukey post hoc test confirmed that activity was significantly different from the baseline after PG (30-60, 150, 240 minutes), CBD 100 mg/mL (30, 90-240 minutes), THC 25 mg/mL (30, 120-240 minutes), and after THC 25 mg/mL + CBD 100 mg/mL (120-240 minutes) inhalation. Activity was significantly lower than the PG inhalation condition following CBD 100 mg/mL (60 minutes), THC 25 mg/mL (60 minutes), and after THC 25 mg/mL + CBD 100 mg/mL (60 minutes) inhalation. There was a significant difference from the CBD 100 mg/mL condition was confirmed 60 minutes after the start of inhalation in the THC 25 mg/mL + CBD 100 mg/mL condition. Activity also differed significantly between the THC 25 mg/mL and THC 25 mg/mL + CBD 100 mg/mL (30-60 minutes) 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 (N=8) and female rats (N=8) 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 randomized order.

Females: The body temperature of female rats was decreased by drug inhalation (**Figure 5**) and the ANOVA confirmed significant effects of Time Post-initiation [$F(8, 56) = 24.01$; $P<0.0001$], of Vapor Condition [$F(3, 21) = 15.3$; $P<0.0001$] and of the interaction of factors [$F(24, 168) = 5.19$; $P<0.0001$]. The Tukey post hoc test confirmed that body temperature was significantly different from the baseline after PG (120-240 minutes), CBD 400 mg/mL (30-240 minutes), THC 100 mg/mL (30-240 minutes), and after THC 100 mg/mL + CBD 400 mg/mL (30-240 minutes) inhalation. Correspondingly, body temperature was significantly lower than the PG inhalation condition following CBD 400 mg/mL (30-180, 240 minutes), THC 100 mg/mL (30-210 minutes), and after THC 100 mg/mL + CBD 400 mg/mL (30-240 minutes) inhalation. In addition, significant differences from the CBD 400 mg/mL condition were

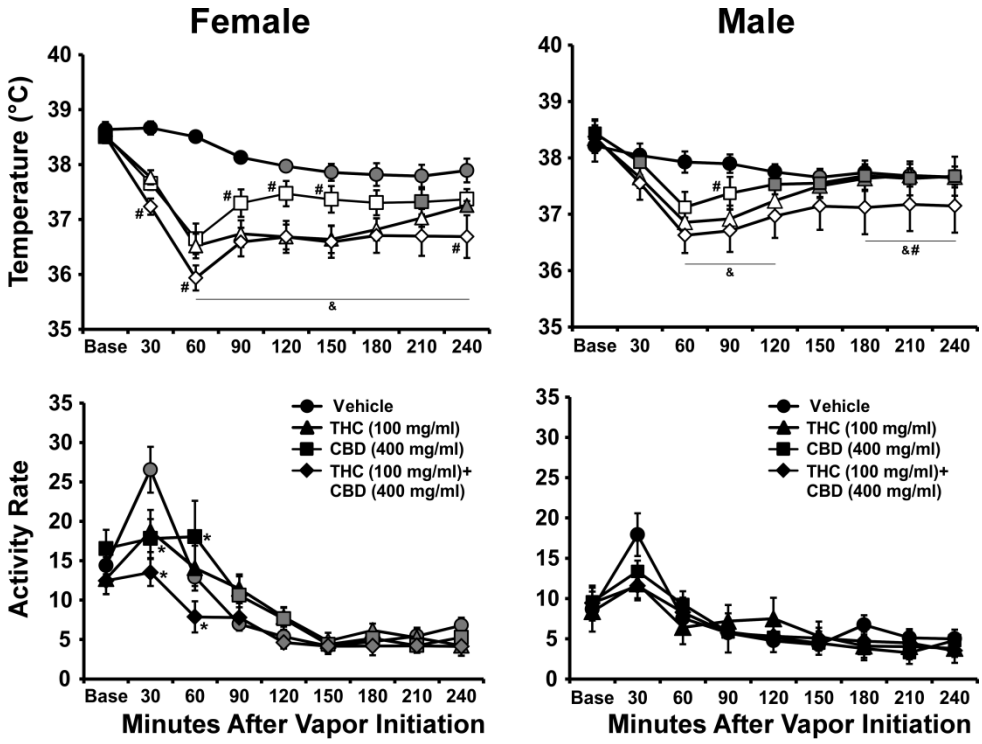

Figure 5: *Mean female (N=8; ±SEM) and male (N=8; ±SEM) temperature and activity following vapor inhalation of the PG, THC (100 mg/mL in PG), cannabidiol (CBD; 400 mg/mL) or the THC/CBD combination. Open symbols indicate a significant difference from both vehicle and the baseline while shaded symbols indicate a significant difference from the baseline only. A significant difference from the vehicle condition 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 &.*

confirmed for THC 100 mg/mL (90-150 minutes), and for THC 100 mg/mL + CBD 400 mg/mL (60-240 minutes) inhalation. Body temperature was also different between the THC 100 mg/mL and THC 100 mg/mL + CBD 400 mg/mL, (30-60, 240 minutes) after inhalation conditions.

The ANOVA confirmed significant effects of Time Post-initiation [$F(8, 56) = 34.82$; $P<0.0001$] and the interaction of Time with Dosing condition [$F(24, 168) = 3.26$; $P<0.0001$] on activity rate. The Tukey post hoc test confirmed that activity was significantly different from the baseline after PG (30, 90-240 minutes), CBD 400 mg/mL (90-240 minutes), THC 100 mg/mL (30, 150-240 minutes), and after THC 100 mg/mL + CBD 400 mg/mL (120-240 minutes) inhalation. Activity was also significantly different than the PG inhalation condition following CBD 400 mg/mL (30-60 minutes) and after THC 100 mg/mL + CBD 400 mg/mL (30-60 minutes) inhalation. A significant difference from the CBD 400 mg/mL condition was confirmed for THC 100 mg/mL+ CBD 400 mg/mL 60 minutes after the start of inhalation. Activity was also significantly different between the THC 100 mg/mL and THC 100 mg/mL + CBD 400 mg/mL, 30-60 minutes after initiation of inhalation.

Males: The ANOVA confirmed significant effects of Time Post-initiation [$F(8, 56) = 12.25$; $P<0.0001$], and of the interaction of factors [$F(24, 168) = 3.64$; $P<0.0001$] on body temperature (**Figure 5**). The Tukey post hoc test confirmed that body temperature was significantly different from the baseline after PG (150, 210-240 minutes), CBD 400 mg/mL (30-240 minutes), THC 100 mg/mL (30-240 minutes), and after THC 100 mg/mL + CBD 400 mg/mL (30-240 minutes) inhalation. Correspondingly, body temperature was significantly lower than the PG inhalation condition following CBD 400 mg/mL (60-90

minutes), THC 100 mg/mL (60-120 minutes), and after THC 100 mg/mL + CBD 400 mg/mL (30-240 minutes) inhalation. In addition, significant differences from the CBD 400 mg/mL condition were confirmed for THC 100 mg/mL (90 minutes) and THC 100 mg/mL + CBD 400 mg/mL (60-120, 180-240 minutes) inhalation. Body temperature was also different between the THC 100 mg/mL and THC 100 mg/mL + CBD 400 mg/mL, (180-240 minutes) after inhalation conditions.

The ANOVA confirmed significant effects of Time Post-initiation [F (8, 56) = 13.86; P<0.0001], but not of Vapor Condition or of the interaction of factors, on activity rate. The marginal mean post hoc test confirmed that across vapor conditions the activity rate was significantly higher than all other time points at 30 min after the start of vapor and significantly lower than baseline activity 150-240 minutes after the start of inhalation.

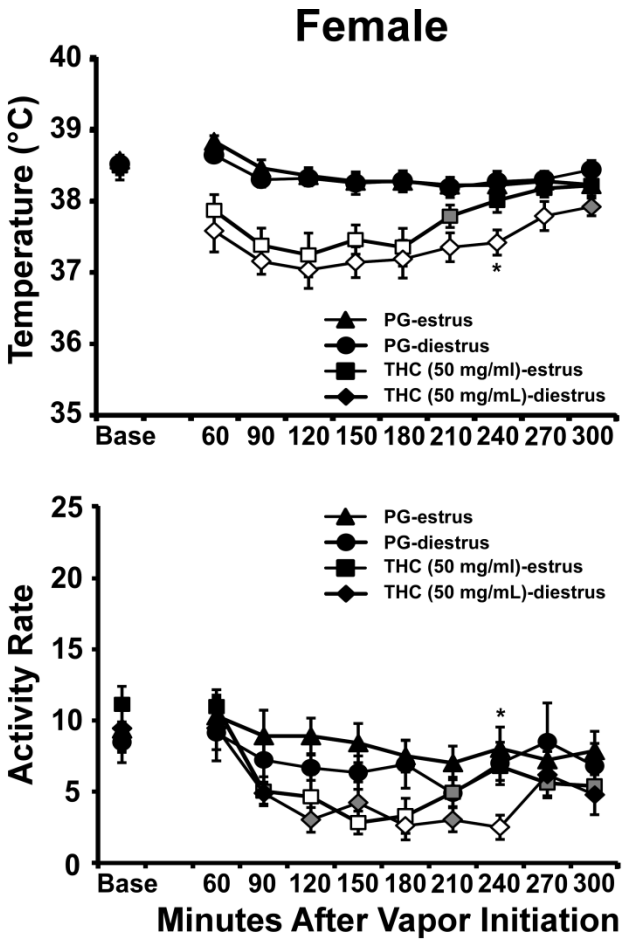

Figure 6: *Mean (N=8; ±SEM) temperature and activity following vapor inhalation of the PG or THC (50 mg/mL in PG) for 30 minutes in estrus and diestrus stages. Open symbols indicate a significant difference from both vehicle and the baseline, while shaded symbols indicate a significant difference from the baseline only; A significant difference between estrus and diestrus phase in THC 50 mg/ml at a given time is indicated by **

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

THC (50 mg/mL; 30 minutes): Mean body temperature was significantly lowered by THC inhalation (**Figure 6**) as confirmed by a significant effect of Drug / estrous condition [F (3, 21) = 19.15; P < 0.0001], Time Post-Initiation [F (9, 63) = 13.12; P < 0.0001], and the interaction of factors [F (27, 189) = 3.42; P < 0.0001]. Body temperature was significantly different from the baseline value after 30 minutes exposure to 50 mg/ml THC (60-210 minutes) for estrus phase and (60-270 minutes) for diestrus phase. The post-hoc test also confirmed that temperature differed from the corresponding vehicle after 30 minutes exposure to 50 mg/ml THC (60-180 minutes) for estrus phase and (60-300 minutes) for diestrus phase. Finally temperature was significantly different between estrus and diestrus phase, after 240 minutes post-exposure to THC 50 mg/ml.

The ANOVA confirmed significant effects of Time Post-initiation [F (9, 63) = 13.80, P<0.0001] and of Vapor Condition [F (3, 21) = 5.00; P<0.01] on activity rate. The Tukey post hoc test confirmed that

activity was significantly different from the baseline after 30 minutes exposure to 50 mg/ml THC (90-210 and 270-300 minutes) for estrus phase and (120-240 minutes) for diestrus phase. The post-hoc test also confirmed that activity differed from the corresponding vehicle after 30 minutes exposure to 50 mg/ml THC (120-180 minutes) for estrus phase and (180, 240 minutes) for diestrus phase. Finally activity was significantly different between estrus and diestrus phase, after 240 minutes post-exposure to THC 50 mg/ml.

THC (25 mg/mL; 40 minutes): In this study N=6 completed the estrus evaluations and N=7 completed the diestrus evaluations due to scheduling constraints (**Figure 7**). The female rats' body temperature was again significantly affected by THC dose condition [F (3, 22) = 5.66; P < 0.01], by Time Post-Initiation [F (9, 198) = 14.94; P < 0.0001] and by the interaction of factors [F (27, 198) = 2.83; P < 0.0001]. The post-hoc test further confirmed that temperature was significantly different from the baseline value after 40 minutes of inhalation 25 mg/ml THC (90-180 minutes) for estrus phase and (90-150 minutes) for diestrus phase. The post-hoc test also confirmed that temperature differed from the corresponding vehicle after 40 minutes of exposure to 25 mg/ml THC (90-180 minutes) for both estrus and diestrus phases. Finally temperature was not significantly different between estrus and diestrus phase within either drug condition. Exposure to THC 12.5 mg/ml for 40 minutes did not affect body temperature and activity in either estrus or diestrus phases.

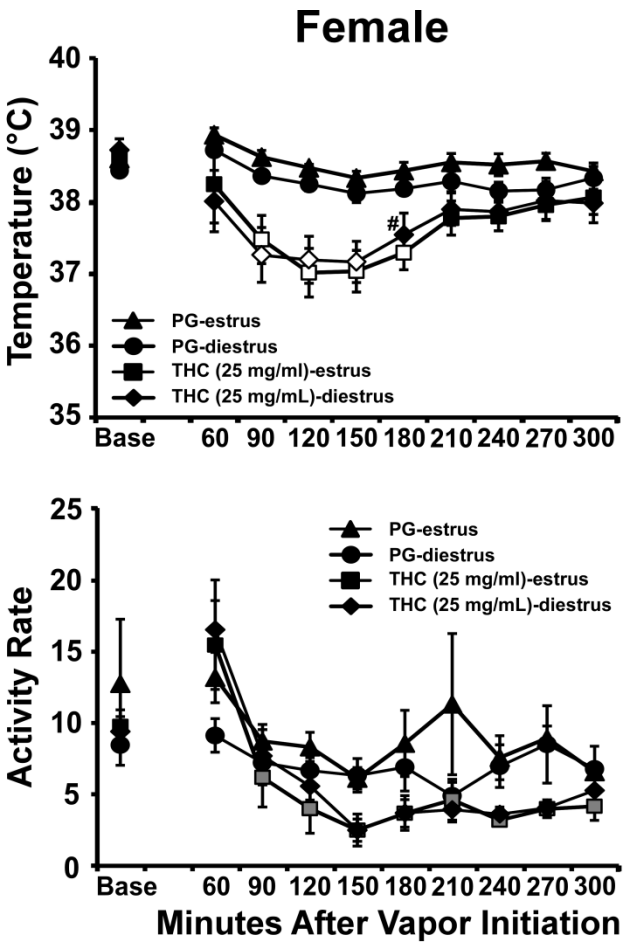

Figure 7: *Mean (N=6-7; ±SEM) temperature and activity following vapor inhalation of the PG or THC (25 mg/mL in PG) for 40 minutes in estrus and diestrus stages. Open symbols indicate a significant difference from both vehicle and the baseline, while shaded symbols indicate a significant difference from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

Activity rate was significantly affected by Time Post-Initiation [F (9, 198) = 14.52; P < 0.0001]. Locomotor activity was significantly different from the baseline value after 40 minutes exposure to 25 mg/ml THC (90-300 minutes) for diestrus phase. Activity rate was not significantly different between estrus and diestrus phase, after exposure to THC 25 mg/ml.

The Hypothermic Effect of THC Inhalation Did Not Change Across Experiments

A further analysis compared the temperature response to 100 mg/mL THC (for 30 minutes) in the first experiment (Time 1) and the high-dose combination study (Time 2). The purpose was to assess any possible plasticity in the response to the inhalation of THC across the repeated dosing conditions within group, as well as to shed light on any possible effects of body mass **(Figure 8)**. The 2-way ANOVA confirmed that body temperature was significantly affected by sex/dose condition [F (3, 28) = 3.01; P<0.05], by Time Post-Initiation [F (7, 196) = 59.42; P<0.0001] and by the interaction of factors [F (21, 196) = 2.63; P<0.0005]. Analysis did not confirm any difference in the *initial* (60-120 min after vapor initiation) male and female body temperature responses to THC within or across the Time 1 and Time 2 experiments. Within group, the female temperature at Time 2 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 at 150-210 minutes post-initiation. 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 weight of the males increased by 66%.

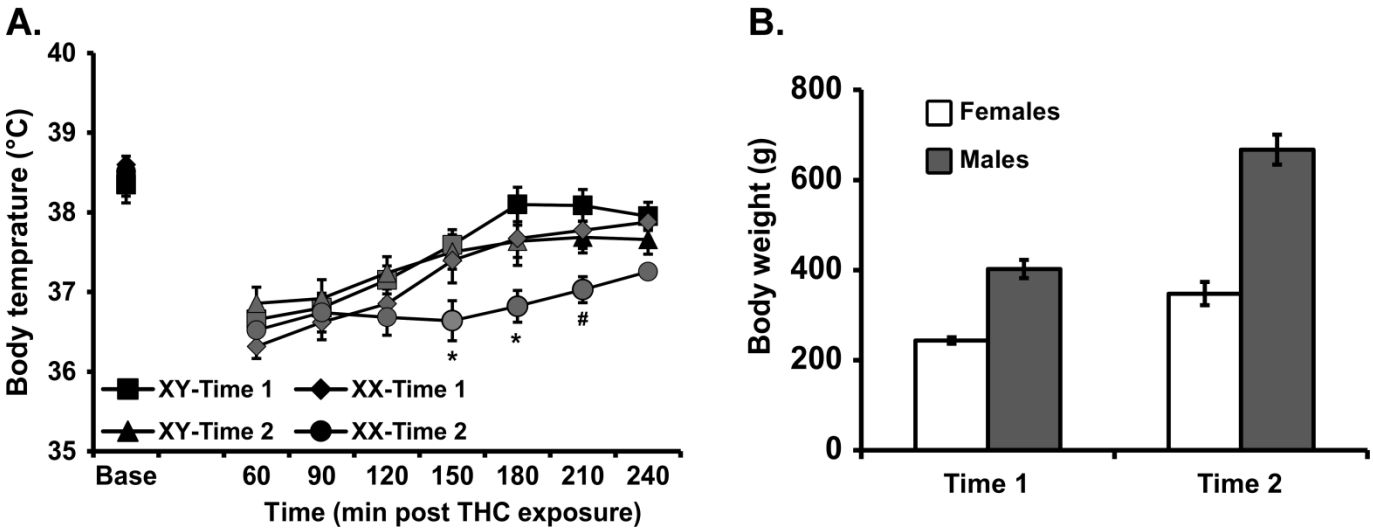

Figure 8: A) *Mean male and female (N=8 per group; ±SEM) temperature following vapor inhalation of THC (100 mg/mL in PG) for 30 minutes in Experiments 1 (Time 1) and 7 (Time 2). Shaded symbols indicate a significant difference from the baseline. A significant difference from all other groups/Times is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

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 9**). The initial 3-way ANOVA confirmed that tail withdrawal latency was significantly affected by sex [F (1, 2) = 16.3; P < 0.0001], THC/PG condition [F (1, 2) = 59.8; P < 0.0001] and water bath temperature [F (2, 2) = 140; P < 0.0001].

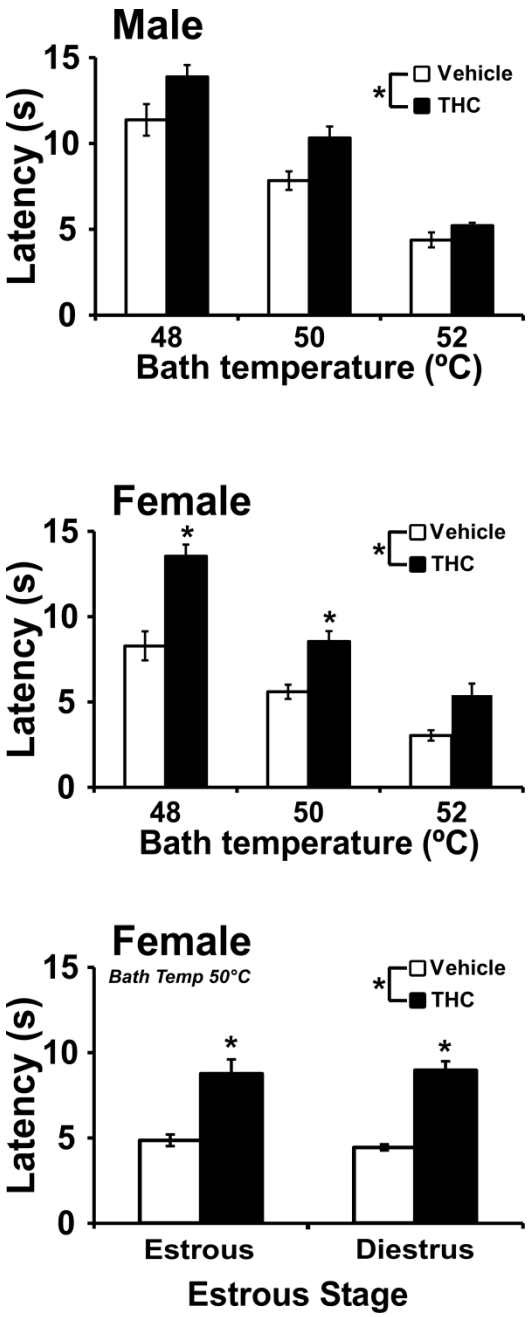

Figure 9: *Mean (N=8; ±SEM)* tail withdrawal latency *following vapor inhalation of the PG or THC (100 mg/mL in PG) for 30 minutes. A significant difference between the vehicle and THC conditions is indicated by *.*

The analysis also confirmed a significant interaction of THC treatment condition and sex [F (1, 2) = 5.01; P < 0.05] and an interaction of water temperature with THC/PG treatment [F (2, 2) = 3.48; P<0.05]. The Tukey post-hoc test including all possible comparisons confirmed that latency was significantly longer after THC inhalation for female rats when evaluated at 48ºC and 50ºC. Significantly longer latencies were confirmed for male versus female rat tail withdrawal from 48ºC water after PG inhalation. Follow-up two-way ANOVA were conducted within sex groups to further parse these effects. This analysis first confirmed significant effects of Water Temperature and Vapor condition, but not the interaction, for female [F (2, 14) = 106.9; P<0.0001, F (1, 7) = 65.27; P<0.0001] and male [F (2, 14) = 168.6; P<0.0001, F (1, 7) = 12.87; P<0.01] rats. The marginal mean post-hoc analysis further confirmed significant differences between PG and THC inhalation, and between all three water bath temperatures for each sex. Since the inhalation conditions were randomized for the full tail-flick study, not all estrus stages were 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 only. In this case the tail withdrawal latency was significantly slowed by THC inhalation, but not estrous stage, as confirmed by a significant effect THC/PG inhalation condition [F (1, 7) = 51.51; P < 0.001] without effect of estrous stage or the interaction of factors. The post-hoc test also confirmed an anti-nociceptive effect of THC in each of the estrus and diestrus phases.

Discussion:

In this study we report the first direct sex comparison following inhalation of cannabidiol (CBD), Δ^9-tetrahydrocannabinol (THC) and their combination in rats using an e-cigarette based vapor technology. THC concentrations was chosen based on our previous study which showed that plasma THC levels

found 30 min after initiation of vapor THC (200 mg/mL) inhalation is 176 ng/ml and 69 ng/mL after 40 min inhalation of a crude extract estimated to contain 116 mg/mL THC in the PG. Although plasma THC concentrations in human vary depending on the potency of marijuana and the manner in which the drug is smoked, studies have found peak THC plasma concentrations of 62-162 ng/mL using ad libitum and paced inhalation procedures (Hartman et al. 2015; Huestis et al. 1992). Thus this model produces THC exposure congruent with human exposure. The results show that male and female rats exhibit similar hypothermic and anti-nociceptive responses to THC when delivered by the inhalation route. This lack of a sex difference did not appear to be related to estrous cycle of the female animals since targeted comparison of hypothermia and anti-nociceptive responses to THC did not show any substantial differences between estrus and diestrus phases. CBD also decreased body temperature when inhaled by itself 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 smaller maximum extent compared with the effects of THC in both sexes. In addition, 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 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 intermittent repeated dosing schedule, since hypothermia produced by 100 mg/mL was identical from the first experiment to the final combination experiment (**Figure 8**).

Although our prior study (Nguyen et al. 2016b) found a significant sex difference with female rats apparently more sensitive to the hypothermic and hypolocomotor effects of THC inhalation, this study did not confirm this difference in terms of initial magnitude of hypothermia. It may be that the former finding was related to a series of i.v. THC challenges completed in both groups prior to the vapor inhalation led to differential plasticity of the hypothermia response. 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 somewhat longer in the female rats (**Figures 1, 4**) which may represent increased female rat sensitivity. Additional experimentation would be required to confirm and to determine if this difference is associated with the female's smaller body size, pharmacokinetic distribution of THC or some other factor.

The lack of a sex difference in hypothermia in these results may only contrast with some prior results because of the dose. 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). 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 estrus 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 phase (**Figures 5, 6**). 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 in male but not female 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 (Tseng and Craft 2001; Wiley 2003). These latter differences occurred across all stages of the estrous cycle of the females, suggesting that hormonal levels were not the primary mediators of these differences (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 (Romero et al. 2002). In accordance with those studies, the present study showed no estrous cycle-dependent differences in THC effects on locomotion activity between females in estrus and diestrus stages.

The present study also confirmed 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. 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 consistent with previous studies in which cannabinoids were more potent in anti-nociception in female than in male rats (Wakley and Craft 2011; Wakley et al. 2014b). In addition, the anti-nociceptive effects of THC inhalation were identical across estrus and diestrus stages in this study, 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 antinociception was not altered by the administration of estradiol, progesterone, or the combination of both hormones (Wakley et al. 2014a) and overall, THC -induced thermal anti-nociception appears not to be sensitive to ovarian hormone modulation (Craft and Leitl 2008; Craft et al. 2012; Tseng and Craft 2001; Wakley and Craft 2011; Wakley et al. 2014a).

Cannabidiol has variously been shown to increase or oppose the behavioral effects of THC on temperature responses when administered by intraperitoneal injection. 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, neither of which conditions appear consistent with the usual human practices involving smoking or vaping of cannabis or cannabis extracts. In a previous study, we found that CBD, when administered (i.p.) either simultaneously or as a pretreatment was ineffective to decrease the effects of THC on activity and thermoregulation and indeed 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 dose 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 also 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 at either threshold (**Figure 4**) or robust (**Figure 5**) 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). This suggests there may be significant differences in the effects of CBD itself that depend on the route of administration. Since CBD-containing e-cigarette liquids are appearing on the market (Peace et al. 2016), a rodent model is of significant use to further evaluate *in vivo* impacts of inhaled CBD in the future.

As one caveat we do not here report the plasma THC levels obtained. It is therefore possible that systematic differences between the sexes in brain THC levels were produced, obscuring a sex effect. This possibility is countered by several observations. The comparison of the effect of identical dosing conditions (100 mg/mL THC) at two different time points across the course of the study shows an identical magnitude of initial hypothermia across sex and across time. The females were 61% as heavy as the males at time 1 and 52% as heavy at Time 2. Females increased in weight by 43% from Time 1 to Time 2 whereas the males increased by 66% and the females at Time 2 were 86% as heavy as the males at Time 1. Since total ventilation of age-matched male and female rats is identical (Doperalski et al. 2008) and they do not differ in cortical blood flow (Roof and Hall 2000), female blood volume is 72% of male blood volume (Probst et al. 2006) and female brain weight is 94% of that of males (Bishop and Wahlsten 1999), if anything the females would have experienced a *higher* effective dose. As reviewed above, where sex differences have been reported following parenteral injection on a mg/kg weight

adjusted basis, female rats tend to be more sensitive than male rats. In this study, no major sex differences were observed across dosing conditions that produced significant dose-dependent effects. Thus it is most parsimonious to conclude that any sex-dependent differences in effective brain concentrations that may have been reached at a given dosing condition were not large enough to significantly oppose the overall conclusion of minimal sex differences in response to inhaled THC.

In conclusion, we have shown that rat sex does not modify the general pattern of hypothermic or hypolocomotive effects of inhaled THC, CBD or their co-administration, nor the anti-nociceptive effects of THC inhalation. In addition, there was no difference in the female rats' response to the inhalation of THC between estrus and diestrus stages across thermoregulatory, activity and nociceptive measures. These results contrast with some prior results reported for parenteral injection of THC or other cannabinoids. 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.

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