NEURAL AND BEHAVIORAL CORRELATES OF EDIBLE CANNABIS-INDUCED POISONING: CHARACTERIZING A NOVEL PRECLINICAL MODEL

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ABSTRACT

Accidental exposure to Δ^9-tetrahydrocannabinol (THC)-containing edible cannabis, leading to cannabis poisoning, is common in children and pets; however, the neural mechanisms underlying these poisonings remain unknown. Therefore, we examined the effects of acute edible cannabis-induced poisoning on neural activity and behavior. Adult Sprague-Dawley rats (6 males, 7 females) were implanted with electrodes in the prefrontal cortex (PFC), dorsal hippocampus (dHipp), cingulate cortex (Cg), and nucleus accumbens (NAc). Cannabis poisoning was then induced by exposure to a mixture of Nutella (6 g/kg) and THC-containing cannabis oil (20 mg/kg). Subsequently, cannabis tetrad and neural oscillations were examined 2, 4, 8, and 24 h after THC exposure. In another cohort (16 males, 15 females), we examined the effects of cannabis poisoning on learning and prepulse inhibition, and the serum and brain THC and 11-hydroxy-THC concentrations. Cannabis poisoning resulted in sex differences in brain and serum THC and 11-hydroxy-THC levels over a 24-h period. It also caused gamma power suppression in the Cg, dHipp, and NAc in a sex- and time-dependent manner. Cannabis poisoning also resulted in hypolocomotion, hypothermia, and anti-nociception in a time-dependent manner and impairments in learning and prepulse inhibition. Our results suggest that the impairments in learning and information processing may be due to the decreased gamma power in the dHipp and PFC. Additionally, most of the changes in neural activity and behavior appear 2 hours after ingestion, suggesting that interventions at or before this time might be effective in reversing or reducing the effects of cannabis poisoning.

INTRODUCTION

Cannabis is mostly consumed through smoking and inhalation [1, 2]; however, the patronage of edible cannabis, a product consumed by ingestion, has increased in recent times [3-5]. This has resulted in an uptick in accidental cannabis poisoning cases among children and pets [6-9], who mistake edible cannabis for other cannabis-free foods [10]. Moreover, edible cannabis is the most common cannabis product associated with cannabis poisoning in children and pets [11-13], emphasizing the need to investigate the effects of edible cannabis-induced poisoning. In children and pets, most clinical signs of cannabis poisoning are neurological [14, 15], occurring due to the interaction between Δ^9-tetrahydrocannabinol (THC) and type 1 cannabinoid receptors (CB1Rs) expressed in brain regions including the cingulate cortex (Cg), prefrontal cortex (PFC), hippocampus, and nucleus accumbens (NAc). The THC in edible cannabis is metabolized into 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) [16], which might be more potent than THC [17, 18] and cause stronger and longer lasting effects [19]. Oral THC administration also results in high brain THC levels [20], which may contribute to the high incidence of edible cannabis-induced poisoning [21].

Intraperitoneal THC administration causes decreased theta and gamma power in the rat hippocampus, a brain region critical for learning [22], possibly through the inhibition of presynaptic neurotransmitter release [23]. Gamma oscillations are necessary for associative learning, and therefore, their alteration may lead to cognitive deficits [24, 25]. THC vapor administration also resulted in decreased gamma power in the rat dorsal striatum, orbitofrontal cortex, and PFC, regions implicated in the cognitive and psychotic effects of THC [26]. While THC inhalation and injection leads to decreased brain oscillations, few studies have investigated similar effects following edible cannabis consumption. Information processing, evaluated as prepulse inhibition (PPI) in the acoustic startle reflex test (ASR) [27, 28], is

impaired in cannabis users [29, 30], and could be used to understand the impact of cannabis poisoning on sensorimotor gating. In rodents, THC administration affects PPI [31-34]. Learning deficits can also be evaluated using the active avoidance task (AAT), and in animal models, acute THC administration causes poor AAT performance [35]. Edible cannabis poisoning may also produce such deficits. While THC exposure via vapor, injection, and gavage induces specific behavioral effects referred to as the cannabis tetrad, which includes catalepsy, hypolocomotion, hypothermia, and anti-nociception [36-38], few studies have evaluated similar behavioral effects using edible cannabis [39, 40]. Sex differences in sensitivity to the effects of THC [41, 42] and its metabolism [43] exist, therefore, it may be worth investigating these differences with respect to edible cannabis poisoning.

This is an important topic given that most patients who report to the emergency unit due to cannabis poisoning report consuming edible cannabis [44]. Understanding the underlying neural mechanisms and the pharmacokinetics of THC via edible consumption could help identify effective treatments for cannabis poisoning. Therefore, the objectives of this study were to investigate the effects of acute high-dose THC via edible administration on neural activity, behavior, and serum and brain THC and 11-OH-THC levels over a 24-h period.

MATERIALS AND METHODS

Detailed description of the study methods can be found in the online Supplementary Material section. Two adult male and female Sprague Dawley rat cohorts were used to develop a novel preclinical model for cannabis poisoning using a commercially available full-spectrum THC cannabis oil. The first cohort underwent surgery to implant electrodes in the dorsal hippocampus (dHipp), PFC, Cg, and NAc followed by neural activity recording and tests to evaluate the cannabis tetrad 2, 4, 8, and 24 h after cannabis poisoning. The second cohort

underwent tests including the ASR to evaluate PPI and AAT to evaluate learning. Saphenous vein blood samples were collected from this group 4, 8, and 24 h after cannabis poisoning for serum THC and 11-OH-THC levels evaluation. They were subsequently euthanized for assessment of brain THC and 11-OH-THC levels 24 h after cannabis poisoning. The timeline for the experiments is described in Fig. S1. All procedures were in accordance with guidelines set by the University of Guelph Animal Care Committee and guidelines in the Guide to the Care and Use of Experimental Animals.

RESULTS

Serum and brain THC and 11-OH-THC levels following edible cannabis-induced poisoning

There was a significant main effect of sex ($F(1,29)=21.05$, $p<0.0001$), but not time after N-THC consumption (time) ($F(2,58)=2.192$, $p=0.1208$) and sex x time interaction ($F(2,58)=0.8209$, $p=0.4451$), on serum THC concentrations. In males, the THC concentration at all time-points were similar (Fig. 1A). In females, the THC concentration at the 4-h time-point was higher than that at the 24-h ($p=0.0463$) time-point. Moreover, the THC concentration in females was higher than that in males at the 4-h time-point ($p=0.0019$), but not the other time-points. There were significant main effects of sex ($F(1,29)=86.00$, $p<0.0001$), time ($F(2,58)=23.86$, $p<0.0001$), and their interaction ($F(2,58)=21.25$, $p<0.0001$) on the serum 11-OH-THC concentration. In males, there were no time-related differences in 11-OH-THC concentration, while in females, the 11-OH-THC concentration at the 4-h time-point was higher than those at the 8-h ($p=0.0220$) and 24-h ($p<0.0001$) time-points (Fig. 1B). Additionally, the 11-OH-THC concentration in females was higher than that in males at the 4-h ($p<0.0001$) and 8-h ($p<0.0001$) time-points, but not the 24-h time-point. Comparison using

the t-test revealed higher brain THC and 11-OH-THC levels in females (p=0.0159 and p=0.0085, respectively) compared to males at the 24-h time-point (Fig. 1C and D).

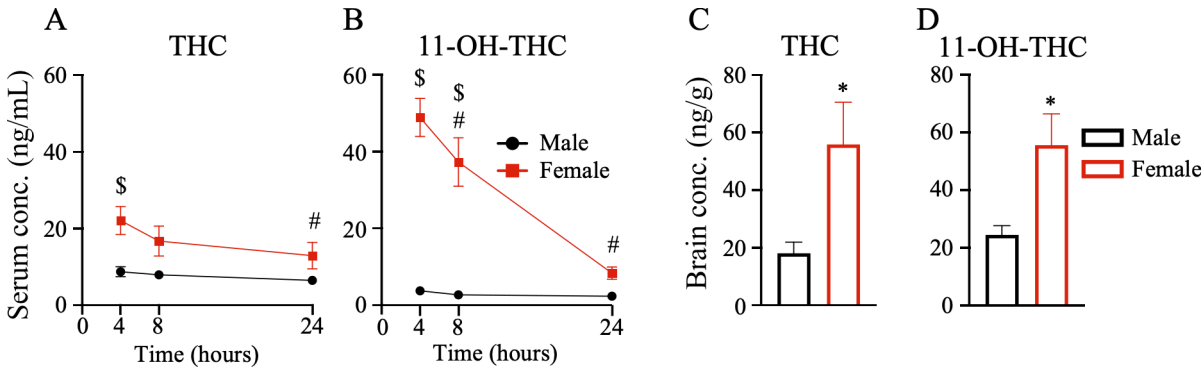

Figure 1. Concentrations of THC and 11-OH-THC in the serum and brain of rats following edible cannabis -induced poisoning. A and B. Serum THC and 11-OH-THC concentrations in males and females at the 4-, 8-, and 24-h time-points. The serum THC and 11-OH-THC concentrations in females were generally higher than those in males. C and D. Brain THC and 11-OH-THC levels in males and females at the 24-h time-point. The brain THC and 11-OH-THC levels in females were higher than those in males. Filled black circles: Male THC/11-OH-THC, filled red squares: female THC/11-OH-THC. $: comparison between males and females at different time-points with p<0.05. #: comparison of concentration at other time-points with that at the 4-h time-point in females with p<0.05. *: comparison of concentration of THC/11-OH-THC in the brain between males and females with p<0.05.

Effects of edible cannabis-induced poisoning on gamma oscillations

Although N-THC consumption had some effect on delta, theta, and beta oscillations, the low and high gamma oscillations were the most consistently affected. Therefore, only the results for the effects of acute high-dose THC via edible administration on PFC, dHipp, Cg, and NAc gamma oscillations at the different time-points (Fig. 2) will be described.

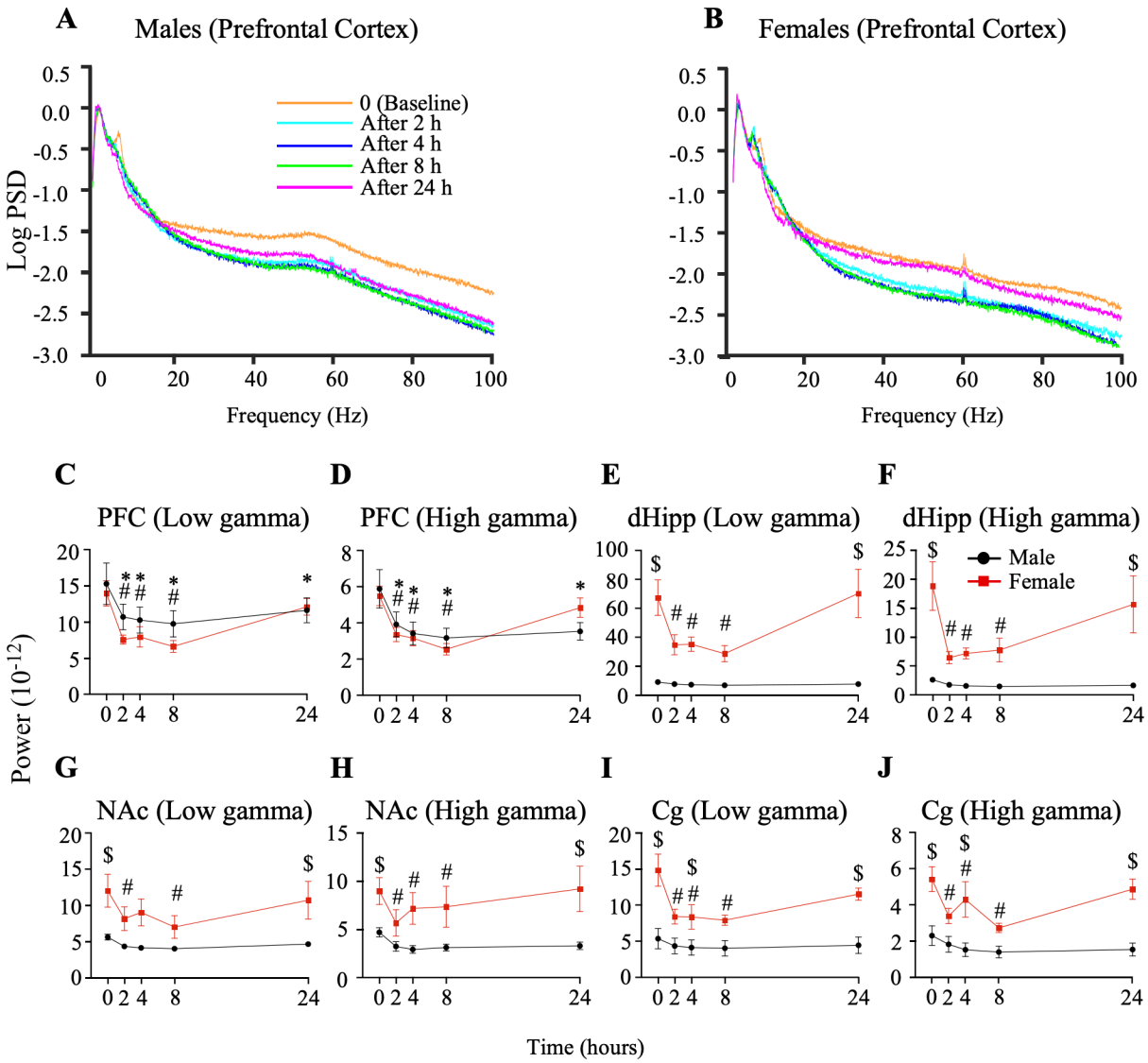

Figure 2. Power spectral density (PSD) plot for male and female rats after edible cannabis-induced poisoning. Representative log-transformed prefrontal cortex (PFC) PSD in male (A) and female rats (B) at each time-point. Orange plot: Baseline, cyan plot: 2-h time-point, blue plot: 4-h time-point, green plot: 8-h time-point, and pink plot: 24-h time-point. C and D. Low and high gamma power, respectively, in the PFC between males and females at different time-points. The low and high gamma power in the PFC of male and female rats were generally not different at the targeted time-points. E and F. Low and high gamma power, respectively, in the dorsal hippocampus (dHipp) of males and female rats at different time-points. G and H. Low and high gamma power, respectively, in the nucleus accumbens (NAc) of males and female

rats at different time-points. I and J. Low and high gamma power, respectively, in the cingulate cortex (Cg) between males and females at different time-points. In general, the low and high gamma power in the dHipp, Cg, and NAc of females were higher than those in males (E – J). $: comparison of time-points between sexes with $p<0.05$, *: comparison of power at other time-points with that at baseline in males with $p<0.05$, #: comparison of power at other time-points with that at baseline in females with $p<0.05$.

In the PFC, while the main effects of sex ($F(1,24)=0.7462$, $p=0.3962$) and its interaction with time ($F(4,96)=1.946$, $p=0.1093$) on the low gamma (LG) power were not significant, that for time ($F(4,96)=17.59$, $p=0.0001$) was significant. Moreover, the LG power was similar in males and females at all time-points (Fig. 2A-D). However, in males the LG power at the 2-h ($p=0.0035$), 4-h ($p=0.0012$), 8-h ($p=0.0003$), and 24-h ($p=0.028$) time-points were lower than that at baseline. In females, with the exception of the 24-h LG power, the 2-h ($p<0.0001$), 4-h ($p<0.0001$), and 8-h ($p<0.0001$) LG power was lower than that at baseline. The main effect of sex on high gamma (HG) power in the PFC ($F(1,24)=0.02193$, $p=0.8835$) was not significant; however, that for the time ($F(4,96)=23.42$, $p<0.0001$) and its interaction with sex ($F(4,96)=3.169$, $p=0.0171$) were significant. Additionally, the PFC HG power at baseline and at all time-points were similar in males and females (Fig. 2A-D). The HG power at the 2-h ($p=0.002$), 4-h ($p<0.0001$), 8-h ($p<0.0001$), and 24-h ($p<0.0001$) time-points were lower than that at baseline in males. In females, the HG power at the 2-h ($p<0.0001$), 4-h ($p<0.0001$), and 8-h ($p<0.0001$) time-points, but not the 24-h time-point, was lower than that at baseline.

Significant main effects of sex ($F(1,24)=24.40$, $p<0.0001$), time ($F(4, 96)=5.540$, $p=0.0005$), and their interaction ($F(4,96)=4.897$, $p=0.0012$) on dHipp LG power were observed. Moreover, while the LG power at baseline ($p<0.0001$) and the 24-h time-point ($p<0.0001$) were higher in

females, it was similar between sexes at the other time-points (Fig. 2E). Furthermore, the LG power at the other time-points were not different from that at baseline in males. In females, however, with the exception of the 24-h LG power, the LG power at the 2-h ($p=0.0007$), 4-h ($p=0.0009$), and 8-h ($p<0.0001$) time-points were lower than baseline. Similarly, the main effects of sex ($F(1,24)=14.38$, $p=0.0009$), time ($F(4,96)=5.5$, $p=0.0005$), and their interaction ($F(4,96)=4.294$, $p=0.0031$) on dHipp HG power were significant. Even though the HG power in both sexes were similar at the 2-h, 4-h, and 8-h time-points, those at baseline ($p<0.0001$) and the 24-h ($p=0.0002$) time-point were higher in females (Fig. 2F). In females, but not males, the baseline HG power was higher than those at the 2-h ($p<0.0001$), 4-h ($p<0.0001$), and 8-h ($p=0.0001$) time-points. The one-way RM ANOVA revealed that in males, the baseline LG power was higher than that at the 4-h ($p=0.0118$) and 8-h ($p=0.0044$) time-points, but not different from those at the 2-h and 24-h time-points (Fig. S2A), while the HG power at the 2-h ($p=0.0011$), 4-h ($p<0.0001$), 8-h ($p<0.0001$), and 24-h ($p<0.0001$) were lower than that at baseline (Fig. S2B).

The main effects of sex ($F(1,24)=8.994$, $p=0.0062$) and time ($F(4,96)=4.122$, $p=0.0041$), but not their interaction ($F(4,96)=1.479$, $p=0.2151$), on NAc LG power were significant. The LG power at baseline ($p=0.0109$) and the 24-h ($p=0.0071$) time-point were higher in females than in males, but not different at the 2-h, 4-h, and 8-h time-points (Fig. 2G). In males, the LG power at all time-points were similar; however, in females, the LG power at the 2-h ($p=0.0130$) and 8-h ($p=0.0009$) time-points were higher than that at baseline, while those at the 4-h and 24-h time-points were not. The sex ($F(1,24)=5.544$, $p=0.0271$) and time ($F(4,96)=7.510$, $p<0.0001$), but not their interaction ($F(4,96)=1.578$, $p=0.1867$), had significant main effects on the NAc HG power. Females had higher HG power at baseline ($p=0.0357$) and the 24-h ($p=0.0384$) time-point than males, but similar HG power to males at the other time-points (Fig. 2H).

Additionally, the HG power at baseline in males was similar to those at other time-points. In females, the HG power at the 2-h ($p=0.0002$), 4-h ($p<0.0001$), and 8-h ($p=0.0002$) time-points, but not the 24-h time-point, were higher than that at baseline. In the male NAc, the one-way RM ANOVA revealed that, with the exception of the 24-h LG power, the LG power at the 2-h ($p=0.0158$), 4-h ($p=0.0028$), and 8-h ($p=0.0018$) time-points were lower than that at baseline (Fig. S2C). The baseline HG power in males was not different from that at the 2-h time-point but higher than those at the 4-h ($p=0.0002$), 8-h ($p=0.008$), and 24-h ($p=0.0002$) time-points (Fig. S2D).

Significant main effects of sex ($F(1,24)=14.69$, $p=0.0008$), time ($F(4,96)=8.707$, $p<0.0001$), and their interaction ($F(4,96)=4.506$, $p=0.0023$) on Cg LG power were observed. Compared to males, the LG power in females were higher at baseline ($p<0.0001$) and the 24-h time-point ($p=0.0002$), but not different at the remaining time-points (Fig. 2I). Moreover, while the LG power at baseline was similar to that at the other time-points in males, the LG power at baseline was higher than that at the 2-h ($p<0.0001$), 4-h ($p<0.0001$), and 8-h ($p<0.0001$) time-points, but not the 24-h time-point, in females. Similarly, there were significant main effects of sex ($F(1,24)=14.92$, $p=0.0007$), time ($F(4,96)=7.109$, $p<0.0001$), and their interaction ($F(4,96)=3.107$, $p=0.0190$) on the Cg HG power. Moreover, while the HG power at the 2-h and 8-h time-points were not different in both sexes, it was lower in males compared to females at baseline ($p=0.0004$) and at the 4-h ($p=0.0048$) and 24-h ($p=0.0001$) time-points (Fig. 2J). Males had similar HG power at all time-points when compared to baseline, while females had HG power at baseline that was higher than that at the 2-h ($p=0.0006$), 4-h ($p=0.0429$), and 8-h ($p<0.0001$) time-points, but not the 24-h time-point. The one-way RM ANOVA revealed that, in males, while the Cg LG power at baseline was not different from that at the other time-points (Fig. S2E), the HG power at baseline was not different from that at the 2-h time-point, but

higher than those at the 4-h ($p=0.0137$), 8-h ($p=0.0344$), and 24-h ($p=0.0320$) time-points (Fig. S2F).

The coherence between pairs of brain regions within the gamma frequency ranges was also evaluated. However, unlike the power spectral density analysis, the results (Fig. S3) were inconsistent and will not be described.

Effects of edible cannabis-induced poisoning on tetrad behavior

While we evaluated the four cannabis tetrad behaviors [38], there were no observable cataleptic effects of cannabis poisoning in both sexes; therefore, results will only be presented for hypolocomotion, hypothermia, and anti-nociception.

There were significant main effects of time ($F(4,44)=26.91$, $p<0.0001$) and sex x time ($F(4,44)=3.295$, $p=0.0191$) on rectal body temperature, with the cannabis exposed animals showing a hypothermia phenotype. Moreover, in males, while the body temperatures at the 2-h ($p=0.0143$), 4-h ($p<0.0001$), 8-h ($p<0.0001$), and 24-h ($p=0.0029$) time-points were decreased compared to that at baseline, in females, the rectal temperatures were decreased at the 2-h ($p=0.0064$), 4-h ($p<0.0001$), and 8-h ($p<0.0001$) time-points, but not the 24-h time-point (Fig. 3A). Additionally, the 24-h time-point rectal temperature of females was higher than that of males ($p=0.014$).

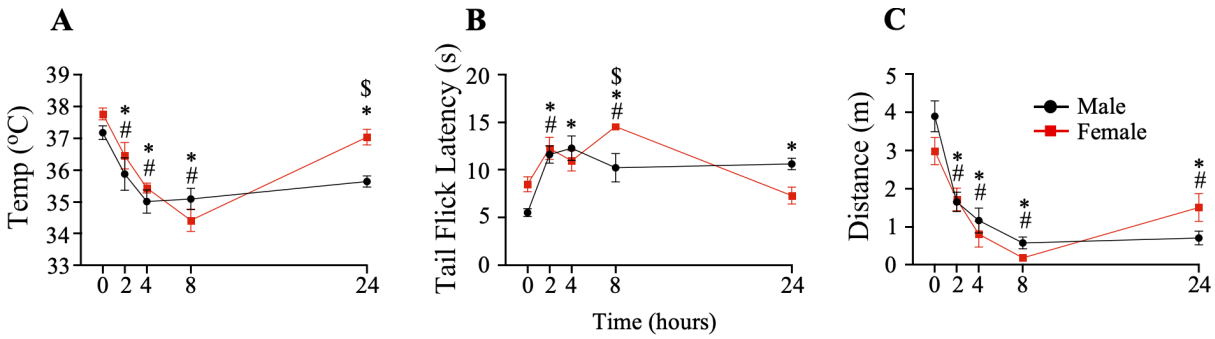

Figure 3. Edible cannabis-induced effects on body temperature, anti-nociception and locomotion in male and female rats. A. Graph comparing the rectal temperatures of males and females measured at the time-points of interest following edible cannabis-induced poisoning. While the rectal temperature at the different time points were different when compared to baseline within male rats and within female rats, the rectal temperature was not different at all time points when male rats were compared to female rats. B. Graph comparing the latency to tail flick of males and females at the time-points of interest following edible cannabis-induced poisoning. While the tail flick latency (anti-nociception) at the different time points when compared to baseline were different within male rats and within female rats, the tail flick latency was not different at all time points when male rats were compared to female rats. C. Graph comparing the total distance traveled by males and females in the open field box at the time-points of interest following edible cannabis-induced poisoning. While the distance traveled in the open field box at the different time points when compared to baseline were different within male rats and within female rats, the distance traveled was not different at all time points when male rats were compared to female rats. $: comparison of outcome at different time-points between sexes with $p<0.05$, *: comparison of outcome at other time-points with that at baseline in males with $p<0.05$, #: comparison of outcome at other time-points with that at baseline in females with $p<0.05$.

There were significant main effects of time ($F(4,44)=11.92$, $p<0.0001$) and sex x time interaction ($F(4,44)=5.517$, $p=0.001$) on edible cannabis-induced anti-nociception. The latency to tail flick in males was longer at the 2-h ($p=0.0003$), 4-h ($p<0.0001$), 8-h ($p=0.0067$), and 24-h ($p=0.003$) time-points compared to baseline. In females, the tail flick latency was longer at the 2-h ($p=0.0209$) and 8-h ($p<0.0001$) time-points but not at the 4-h and 24-h time-points (Fig. 3B). Moreover, the tail flick latency of females was longer compared to males after 8 h ($p=0.0091$) and tended to be shorter than that in males at the 24-h ($p=0.0743$) time-point.

There was a significant main effect of time on displacement (distance traveled) ($F(4,44)=32.90$, $p<0.0001$), with animals showing hypolocomotion after edible cannabis exposure. However, the main effect of sex ($F(1,11)=0.5725$, $p=0.4652$) was not significant, while that for the sex x time interaction ($F(4,44)=2.453$, $p=0.0598$) trended toward significance. Additionally, compared to displacement at baseline, the displacement of both males and females at the 2-h (male: $p<0.0001$, female: $p=0.0092$), 4-h (male: $p<0.0001$, female: $p<0.0001$), 8-h (male: $p<0.0001$, female: $p<0.0001$), and 24-h (male: $p<0.0001$, female: $p=0.0019$) time-points were decreased (Fig. 3C).

Effects of edible cannabis-induced poisoning on active avoidance learning and prepulse inhibition

In the AAT, the three-way ANOVA revealed significant main effects of trial block ($F(4.322,109.5)=30.14$, $p<0.0001$), treatment (control vs THC groups; $F(1,26)=15.94$, $p=0.0005$), trial block x treatment interaction ($F(6,152)=10.29$, $p<0.0001$), and trial block x sex interaction ($F(6,152)=4.318$, $p=0.0005$) on percentage avoidance. There were no significant main effects of sex, sex x treatment interaction, or trial block x treatment x sex interaction on percent avoidance. We subsequently performed a two-way ANOVA in males which revealed

significant main effects of trial block (F(6,78)=7.037, p<0.0001), treatment (1, 13)=4.701, p=0.0493), and their interaction (F(6,78)=3.396, p=0.0050) on the percentage avoidance (Fig. 4A). The percentage avoidance in control rats during trial blocks 5 (p=0.0474) and 7 (p=0.0098) was higher than that in THC rats but not different during trial blocks 1, 2, 3, 4, and 6. In females, the two-way ANOVA revealed significant main effects of trial block (F(6,74)=27.47, p<0.0001), treatment (F(1,13)=12.79, p=0.0034), and their interaction (F(6,74)=7.552, p<0.0001) on the percentage avoidance. While the percentage avoidance in female control rats during trial blocks 1, 2, 3, and 4 was not different from that for female THC rats, it was higher than that of female THC-exposed rats during trial blocks 5 (p<0.0001), 6 (p=0.0004), and 7 (p=0.0001) (Fig. 4B).

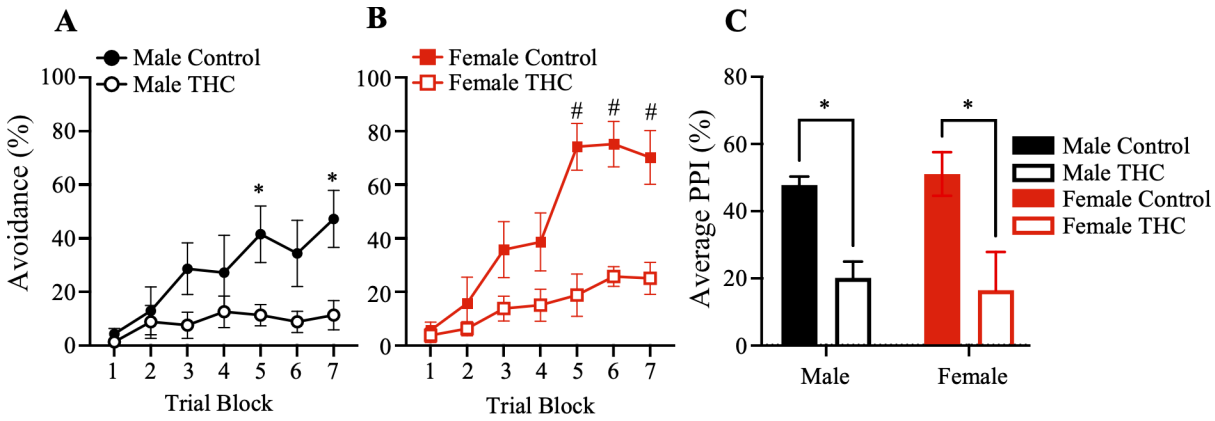

Figure 4. Both active avoidance and prepulse inhibition are disrupted by edible cannabis poisoning. A. Graph comparing the percentage avoidance among male control and THC rats. The percentage avoidance of male control rats was higher than that of male THC rats during trial blocks 5 and 7. Filled black circle: male control rats, empty black circle: male THC rats. B. Graph comparing the percentage avoidance among female control and THC rats. The percentage avoidance of female control rats was higher than that of female THC rats during trial blocks 5, 6, and 7. Filled red square: female control rats, empty red square: female THC rats. *: comparison of percentage avoidance for different trials between male control rats and

male THC rats with $p<0.05$, #: comparison of percentage avoidance for different trials between female control rats and female THC rats with $p<0.05$. C. Graph comparing average percentage PPI among male and female control and THC rats. The average percentage PPI in both male and female THC rats was lower than those of their corresponding control rats. Filled black bar: male control rats, empty black bar: male THC rats, filled red bar: female control rats, empty red bar: female THC rats. *: comparison between control and THC rats with $p<0.05$.

During the two-way ANOVA analysis of the percentage PPI, no difference in the effect of sound intensity on the percentage PPI was observed in both sexes (results not shown); the percentage PPI for the three intensities were therefore averaged and analyzed. While the main effects of sex ($F(1,14)=0.001341$, $p=0.9713$) and sex x treatment interaction ($F(1,14)=0.3888$, $p=0.5429$) on percentage PPI were not significant, that for treatment was significant ($F(1,14)=13.92$, $p=0.0022$). Moreover, in both sexes, the averaged percentage PPI for control rats were higher than that for THC exposed rats (male: $p=0.0209$, female: $p=0.0037$) (Fig. 4C).

DISCUSSION

In this study, we established and characterized a rat model of high-dose edible cannabis poisoning. We observed higher serum and brain THC and 11-OH-THC levels in female rats compared to male rats after cannabis poisoning despite exposure to the same N-THC doses. The study also revealed sex- and time-dependent decreases in dHipp, Cg, and NAc gamma power over a 24-h period starting 2 h after cannabis poisoning. Time-dependent changes in the cannabinoid tetrad behaviors and active avoidance and PPI disruptions were also observed.

The observed sex difference in serum THC levels could be partly due to the rapid redistribution of the lipophilic THC to fatty tissues to a greater extent in males [45-47], who have more body

fat than females [48]. Similar to a previous study [49], we found sex differences in serum 11-OH-THC levels, suggesting differences in THC metabolism, possibly due to the sexually dimorphic expression of cytochrome P450 (CYP) enzymes [50, 51], resulting in THC being preferentially metabolized into 11-nor-9-carboxy-Δ^9-THC (11-COOH-THC) in males and into 11-OH-THC in females [52-54], leading to higher serum 11-OH-THC levels in females. We also observed lower brain THC and 11-OH-THC levels in males compared to females, which may be related to the lower serum levels of the parent compound in males. Further, male rat brains may be protected against THC through increased expression of blood brain barrier proteins (including claudin-5) [53, 54]. The rat brain expresses CYP enzymes that can also metabolize THC [55], suggesting that microsomes in female rats may metabolize more THC into 11-OH-THC, resulting in higher brain 11-OH-THC levels [43]. In general, the serum and brain 11-OH-THC levels decreased gradually over 24 h; while this may be due to its excretion [56], it could be because the 11-OH-THC is rapidly oxidized into 11-COOH-THC [57].

The local field potential power reflects the extent of neural synchrony, with higher power reflecting higher synchrony and vice versa [58]. Gamma power and synchrony is modulated by parvalbumin-expressing (PV) interneurons [59], which are modulated by cholecystokinin-expressing (CCK) interneurons [60], the only CB1R-expressing interneuron in the cortex and hippocampus [61-63]. THC binding to CCK interneuron CB1Rs affect PV interneuron activity, resulting in a disruption in gamma oscillations, and a subsequent gamma power decrease. This may explain the decreased gamma power observed following edible cannabis-induced poisoning in our rats [26, 64-66]. That these decreases in gamma power were observed in the dHipp, Cg, NAc, and PFC, is not surprising since these regions have high CB1R densities. In our study, significant edible cannabis-induced poisoning effects on gamma power began around the 2-h mark, which is consistent with the finding that the subjective behavioral effects

of edible cannabis peak between 1.5 and 3 h after ingestion [67, 68], as well as our pharmacokinetic findings. We also observed sex differences in NAc, Cg, and dHipp gamma power. This may be partly attributable to the high estrogen levels in female rats [69, 70], which increases the binding of estrogen to estrogen β receptors expressed by PV interneurons, leading to increased firing, greater inhibition, and increased gamma activity [71, 72]. This may explain the higher gamma power in female rats, which may also underlie the quicker recovery from the THC-induced suppression in females, where the 24-h time-points no longer showed THC effects.

The similarity in effects of THC on anti-nociception (tail flick latency) in males and females observed in our study was also reported in rats following THC vapor administration [39]. One of the most cited reasons for medical cannabis use is pain relief [73, 74]. The periaqueductal gray, a brain region that contains CB1R-expressing somatodendritic structures and presynaptic terminals [75-77], is involved in pain modulation [78], and may play a role in the decreased pain sensitivity we observed following edible cannabis-induced poisoning. Interestingly, the tail flick latency in female rats had returned to baseline levels, unlike that in male rats, 24 h after acute high-dose THC administration. We also observed edible cannabis-induced hypothermia in both males and females, which may be due to the interaction between THC and the preoptic area of the hypothalamus, a region involved in temperature regulation [79] and known to contain high densities of CB1Rs [80-82]. In female rats, both anti-nociception and body temperature had returned to baseline levels by the 24-h time-point, which could be due to the development of tolerance to the higher serum and brain THC and 11-OH-THC levels or the differential time-course of effects between the parent compound and metabolite. While no sex-differences in hypolocomotion was observed in our study, similar to the findings of a previous study [83], the hypolocomotion, which was also previously reported [84, 85], may be

due to the interaction between THC, 11-OH-THC, and CB1Rs on cerebellar basket cells [86]. Interestingly, the edible cannabis-induced tetrad effects began around the 2-h time-point, similar to the observed neural effects of cannabis poisoning in this study, which coincides with the time-point that cognitive and psychomotor deficits were observed in humans following edible cannabis ingestion [68]. This suggests that interventions to reverse the effects of edible cannabis-induced poisoning should target the 2-h time window.

The AAT and ASR were performed just before the 4-h time-point because the effects of edible cannabis on cognitive performance in humans were observed between 2 and 5 h post-ingestion [68]. We observed decreased hippocampal gamma power, which is necessary for learning the association between stimuli [25, 87, 88]. This could explain the inability of N-THC-treated rats to avoid the foot shock during the AAT. Our finding is consistent with those of previous studies that found learning deficits in cannabis users [89] and THC-treated rats [35]. While low dose THC (0.3 – 3 mg/kg) administration had no effect on PPI [90], a higher dose (10 mg/kg) disrupted PPI [31], similar to our findings in rats that received acute high-dose THC (20 mg/kg). This suggests the possibility of a dose-dependent effect of THC on PPI. A previous study reported decreased PPI in rats following the direct infusion of CB1R agonists into the hippocampus and PFC [34] and concluded that hippocampus and PFC CB1R activation modulates neural GABA and glutamate release, which affects the activities of PPI-mediating structures like the NAc and ventral tegmental area [34]. This conclusion is consistent with our finding of decreased PFC and dHipp gamma power following acute high-dose THC administration, which may have influenced NAc activity.

Although we report several novel findings, there are a number of limitations that are noteworthy. Firstly, we could only administer the high-dose THC once, since the rats consume

the Nutella voluntarily, but do not consume it after exposure to N-THC. Secondly, blood sampling to determine serum THC and 11-OH-THC levels began at the 4-h time-point, just after the AAT and ASR. This was done to ensure that the behavioral effects of the N-THC exposure would not be impacted by the blood sampling, but as a result we may have missed the peak THC and 11-OH-THC levels. Thirdly, although male rats metabolize THC preferentially into 11-COOH-THC, we did not evaluate serum and brain 11-COOH-THC levels. This will be done in future studies. Fourthly, while we chose to use full-spectrum THC oil to more closely mimic human edible cannabis products, we are unable to assess the impact of additional cannabinoids and terpenes in the cannabis oil on the outcomes measured here. Future studies exploring different types of cannabis oils with varying cannabinoid and terpenoid levels may help to clarify these effects. Fifthly, the estrous cycle phase was not assessed in the female rats, which may have impacted the behavioral and electrophysiological effects of edible cannabis. Future studies will be adequately powered to assess the impact of estrous cycle on cannabis poisoning. Lastly, while the model proposed here will be valuable, these findings will need to be extended to younger rats to more closely model cannabis poisoning in children.

In conclusion, edible cannabis-induced poisoning results in decreased gamma power, causes hypolocomotion, hypothermia, and anti-nociception, leads to learning deficits, and impairs PPI. While it is not surprising that cannabis poisoning has neural and behavioral effects, this is the first study to show these effects using edible cannabis, which is increasingly becoming popular among cannabis users and is the most cited cannabis product associated with cannabis poisoning, especially in children and pets. Interestingly, the neural and behavioral effects of edible cannabis-induced poisoning begun after 2 h, which coincides with the period when the peak effects of edible cannabis on cognitive processes were reported. This suggests that

administering interventions during this time window may be key to reversing the effects of cannabis poisoning. Moreover, the sex differences in gamma power and serum and brain THC and 11-OH-THC levels following cannabis poisoning suggests sex differences in the effects of THC and its metabolism, which should be considered for the development of effective treatments.

AUTHOR CONTRIBUTIONS

RQA contributed to the design of the work; acquisition, analysis, interpretation of the data; and drafting and revising the manuscript. HK contributed to the design of the work, acquisition of the data, and revising the manuscript. MAT contributed to the acquisition of the data. AH contributed to the acquisition of the data. YG contributed to the acquisition, analysis, and interpretation of the data. RJ contributed to the acquisition, analysis, and interpretation of the data. KU contributed to the conception of the work, interpretation of the data, and revision of the manuscript. JYK contributed to the conception and design of the work, analysis and interpretation of the data, and the drafting and revision of the manuscript. All authors have approved the final version of the manuscript to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

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COMPETING INTERESTS

Dr. Urban is an employee of Avicanna Inc., during which time she has received stock options. Avicanna Inc. did not influence the design, conduct, or interpretation of the data derived from this study. None of the other authors have any disclosures.

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