A preclinical model of THC edibles that produces high-dose cannabimimetic responses

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Abstract (248 words)

Background: No preclinical approach enables the study of voluntary oral consumption of high dose Δ^9-tetrahydrocannabinol (**THC**) and its intoxicating effects, mainly owing to the aversive response of rodents to THC that limits intake. Here we developed a palatable THC formulation and an optimized access paradigm in mice.

Methods: THC was formulated in chocolate gelatin (**THC-E-gel**). Adult male and female mice were allowed *ad libitum* access for 2 h. Cannabimimetic responses (hypolocomotion, analgesia, and hypothermia) were measured following access. Levels of THC and its metabolites were measured in blood and brain samples. Acoustic startle responses were measured to investigate THC-induced psychotomimetic behavior.

Results: Access to high-dose THC-E-gel (≈30 mg/kg over 2 h) resulted in robust consumption and CB_1 receptor-dependent behavioral responses. High-dose THC-E-gel consumption resulted in parallel accumulation of THC and its psychoactive metabolite 11-OH-THC in brain, a profile that contrasts with the known rapid decline in brain 11-OH-THC levels following intraperitoneal THC injections. High-dose THC-E-gel consumption increased the acoustic startle response preferentially in males, and this psychotomimetic response was remarkably different from the response triggered by intraperitoneal contingent administration of THC. Comparing cannabimimetic responses elicited by intraperitoneal versus oral administration enabled us to model a “predicted dose” of THC that triggers these responses.

Conclusion: Voluntary consumption of high-dose THC-E-gel triggered equivalent cannabimimetic responses in male and female mice but an increased acoustic startle response preferentially in males. These findings indicate that THC-E-gel offers a robust preclinical consumption model to study cannabimimetic responses in mice, including sex-dependent psychotomimetic responses.

Introduction

The use of cannabis products containing high concentrations of THC is rapidly increasing; yet we still have a limited understanding of its possible impact on physical and mental health (1-3). These products are typically inhaled as combusted plant matter, vaporized extracts or increasingly ingested in edible forms. THC acts as a partial agonist at CB_1 receptors (**CB_1R**). THC activates CB_1R and triggers physiological responses (e.g., increase heart rate), alters mood and time perception, and impairs learning and memory (4-8). Alarmingly, use of high-dose THC is associated with acute psychotic episodes and hallucinations (6, 9, 10). In rodents, THC reduces spontaneous locomotion, induces hypersensitivity to tactile and auditory stimuli, ataxia, and sedation; all of which have been shown to be mediated through action at CB_1R (11-13). Importantly, some cannabimimetic responses are sex-dependent, as exemplified by the finding that THC (5 mg/kg, *i.p.*) triggers a more pronounced reduction in spontaneous locomotion and anxiogenic response in females than in males (14, 15). In addition to these cannabimimetic responses, preclinical investigations have pursued psychosis-related behaviors through traditional psychotomimetic responses such as acoustic startle behavior (16, 17), emphasizing the translational value in understanding THC's bioactivity in humans.

Voluntary oral consumption of THC can be achieved by rodents, but results in mild acute CB_1R-dependent cannabimimetic responses due to aversion to higher doses of THC (18-20). Thus, preclinical models of voluntary administration of high-dose THC have proven difficult to establish. This premise emphasizes the urgent need to develop and fully characterize a novel experimental approach to study the impact of high-dose THC. To bridge this translational gap, we initially developed an approach where mice are given *ad libitum* access to consume a sugar-water gelatin (**CTR-gel**) containing fixed amounts of THC (19, 21). Matching previous

rodent studies, we found that mice consumed more vehicle gelatin than THC gelatin, indicating that they detected and avoided THC (22). To overcome this limitation, in the current study we developed and characterize a palatable oral formulation that increases voluntary consumption by formulating THC in a chocolate-flavored nutritional shake, Ensure™ (**E-gel**). Previous work has shown that mice have a preference for chocolate flavor, making it an ideal THC formulant to increase palatability (23, 24). We leveraged this approach to determine whether oral consumption of high THC doses induce commonly studied cannabimimetic responses in mice (hypolocomotion, analgesia, and hypothermia) and then we examined the effects of E-gel THC consumption on acoustic startle response, a preclinical measure of psychotomimetic responses (17, 25). The model developed here leverages acute voluntary consumption of a sweetened gelatin to investigate sensitive psychotomimetic behaviors, pharmacokinetics, and triad responses following consumption of high-dose THC in mice.

Methods and Materials

Animal Studies

Animal studies followed the guidelines established by AAALAC and were approved by IACUC of the University of Washington. Specifics on animals in Supplementary Methods.

Pharmacological Agents

Animals received THC (0.1, 0.3, 1, 3, 5, 10, and 30 mg/kg) and SR141716 (**SR1**, 1 mg/kg) *i.p.* or were exposed to THC suspended in gelatin. Specifics in Supplementary Methods.

Gelatin formulation

All gelatin was formulated following previously published procedures with a substitution of the water- Polycal™ sugar solvent in CTR-gel for dark chocolate Ensure™ in E-gel. Specifics in Supplementary Methods.

Triad of Cannabimimetic behaviors

Hypolocomotion, hypothermia, and analgesia were measured 1 h post-*i.p.* injection or immediately following gelatin exposure. Pre-tests were collected immediately prior to injection or gelatin exposure. Specifics in Supplementary Methods.

Histology

Animals underwent the same gelatin exposure paradigm for day 1 and 2. Blood and brain samples were collected at 4 time points. Specifics in Supplementary Methods.

Acoustic startle

Acoustic Startle behaviors were measured after 1 or 2 h THC-E-gel exposure (10 mg/15 ml) and THC-*i.p.* (0.1, 1, 5, 10 mg/kg) injection. Specifics in Supplementary Methods.

Data analysis

All data were analyzed using GraphPad Prism 10-11. All behavioral locomotor tracking was analyzed using Noldus Ethovision software. For all statistical analyses (unpaired *t* test, one- and two-way ANOVA, and post hoc analyses), alpha level was set to 0.05.

Results

E-gel promotes heightened voluntary oral consumption of THC by adult mice.

To incentivize voluntary oral consumption of high-dose THC, we utilized an E-gel formulation and optimized access paradigm based on previous studies (19, 26). Here, individual mice were exposed to (control, **CTR-gel**) during a 2 h consumption period (Habituation, Day 1); and the following day exposed to THC formulated in either CTR-gel or E-gel (Xmg/15 ml) for 2 h (**Figure 1a**). Gelatin mass was measured before and after access to calculate grams consumed, and gelatin concentrations are expressed as *X* mg of THC (X = mg of THC/15 ml gelatin) (**Figure 1b**). As expected, high-dose THC-CTR-gel reduced consumption, an effect

significant at 1 mg THC (**Figure 1c**). Remarkably, 7/17 (41%) of mice exposed to 4 mg CTR-gel did not consume any gelatin while all mice consistently consumed 5 mg and 10 mg THC-E-gel (**Figure 1d**). Thus, mice consumed 1.9 ± 0.05 g of CTR-E-gel and 1.0 ± 0.07 g of E-gel containing THC (10 mg/15 ml) (**Figure 1d**). Note that mice consumed similar amounts of VEH-E-gel and VEH-CTR-gel (1.96 ± 0.15 g and 1.92 ± 0.17 g, respectively), indicating that chocolate flavor *per se* does not increase consumption. When calculating the amount of THC consumed in mg/kg, we found that mice consumed more THC when formulated in E-gel (**Figures 1e**). For example, mice consumed 10.5 ± 0.7 mg/kg/2 h when exposed to 2 mg/15 ml E-gel compared to only 4.6 ± 0.5 mg/kg/2 h when exposed to the same amount of THC formulated in CTR-gel. Using this experimental approach, maximal consumption reached 29.2 ± 1.8 mg of THC *per* kg over 2 h when exposed to E-gel containing THC (10 mg/15 ml), compared to the few mice that consumed only 8.4 ± 1.2 mg of THC *per* kg over 2 h when exposed to CTR-gel containing THC (4 mg/15) (**Figures 1e**). As previously shown, we found no statistical difference in consumption between male and female mice (19, 21) (Supplementary Figure S1). These data show that mice consistently consume significant quantities of E-gel despite high THC concentrations. Based on these results, we next focused our study on quantifying the pharmacological effects of THC formulated in E-gel.

High-dose THC reduces locomotion during the exposure period.

We found that mice consumed ~2 g of vehicle E-gel (VEH-E-gel) compared to ~1 g of high-dose THC-E-gel (10 mg/15 ml), indicating a 2-fold reduction in consumption (**Figure 1d**). To investigate the time course of this effect, we weighed gelatin every 10 min during the 2 h access period in a 3-day paradigm: access to VEH-E-gel on Day 1, access to either VEH-E-gel or THC-E-gel Day 2, and access to VEH-E-gel on Day 3 (**Figure 2a**). **Figure 2b** shows that

consumption of VEH-E-gel on Day 1 started within 20 min of availability and was constant during the 2 h period. On Day 2, mice consumed comparable amounts of VEH-E-gel and THC-E-gel during the initial 40 min of access (16.3 and 13.0 mg/min, respectively) (**Figure 2c** and Supplementary Figure S2a). However, consumption of THC-E-gel plateaued after 40 min to a rate of 4.2 mg/min (67.7% reduction), while consumption of VEH-E-gel was sustained at 9.9 mg/min (39.3% reduction) (**Figure 2c** and Supplementary Figure S2b). By sharp contrast, mice that had consumed THC-E-gel the day prior consumed VEH-E-gel on Day 3 at a significantly slower rate (6.2 mg/min), suggesting an aversive memory to THC-E-gel (**Figure 2d** and Supplementary Figure S2b). Mice that had consumed THC-E-gel the day prior also delayed their consumption of at least 0.2 g of VEH-E-gel on Day 3 by 3-4-fold (Supplementary Figure S2c). Consequently, mice exposed to THC-E-gel on Day 2 decreased their total VEH-E-gel consumption on Day 3 by approximately half (**Figure 2e**). The combination of decreased rate of consumption and increased latency to consume VEH-E-gel on Day 3 suggests that, on Day 2, mice consumed high enough quantities of THC to induce a high-dose cannabimimetic response resulting in an avoidance to gelatin on Day 3.

Reduced spontaneous locomotion is a hallmark response to THC in mice. To address whether THC-E-gel consumption impacts spontaneous locomotion, we video-recorded the travelling distance of mice during the gelatin access period (total distance in cm over 2 h) (**Figure 2f**). **Figure 2g** shows that locomotion during the consumption period initially reached (1,200 cm/ 5 min) and then steadily decreased over the 2 h session on Day 1, as expected in mice that are habituating to the environment. On Day 2, spontaneous locomotor between mice that consumed VEH-E-gel and THC-E-gel diverged after 40 min, showing a significant decrease in total locomotion in mice that consumed THC-E-gel (**Figure 2h**). Thus, this

reduction in locomotion parallels a corresponding reduction in consumption (**Figure 2c**). Importantly, spontaneous locomotion of mice exposed to VEH-E-gel and THC-E-gel was similar on Day 3. Together, these results show that consumption of THC-E-gel induced hypolocomotion on Day 2 after 40 min of access. Additionally, the decreased consumption of VEH-E-gel on Day 3 is likely due to an aversive memory to THC and not to hypolocomotion. Thus, E-gel incentivizes voluntary THC consumption to induce robust hypolocomotion, a hallmark cannabimimetic response, within 40 min of access.

THC-E-gel consumption triggers CB_1R-dependent behaviors.

Considering that THC-CTR-gel triggers mild cannabimimetic responses due to limited consumption (19, 21), we determined whether consumption of THC-E-gel could induce cannabimimetic responses measured immediately following the 2 h access period (**Figure 3a**). Thus, we selected three well-described behavioral effects of THC in mice: hypolocomotion, analgesia, and hypothermia (**Figure 3b-d**) (13). THC was formulated and administered either by *i.p.* injection (grey), CTR-gel (green), or E-gel (purple) and behavioral responses to gelatin consumption were plotted from the average dose consumed, calculated in **Figure 1e**. **Figure 3b** shows that *i.p.* administration of THC reduced locomotion starting at 3 mg/kg, as previously reported (13), and that this response was significant after access to 4 mg THC-CTR-gel and 2 mg THC-E-gel (Supplementary Figure 3b, e). **Figure 3b** also shows that this hypolocomotion response was significant at 4.64 mg/kg THC-CTR-gel (16.8%) and following access to 10 mg THC-E-gel (52.4%). **Figure 3c** shows that maximal THC-induced analgesia was reached at 30 mg/kg *i.p.,* after access to 2 mg. *i.p.* injection of THC reduced core body temperature starting at 3 mg/kg, and that this response reached significance at 4 mg THC-CTR-gel and at 5 mg THC-E-gel (**Figure 3d** and Supplementary Figure S3d, g). **Figure 3d** also shows that reduced

core body temperature induced by THC reached a maximum of -5.84°C at 30 mg/kg *i.p.*, -1.5°C after 4 mg THC-CTR-gel and -1.8°C after 10 mg THC-E-gel. Analgesia and hypothermia did not plateau, matching prior studies which have also shown that 30 mg/kg THC-*i.p.* does not produce a maximal response for these cannabimimetic behaviors (27, 28). Thus, **Figures 3b-d** show that: 1) low dose THC reduces locomotion to the same extent when administered using these three experimental paradigms, and to a greater extent at high dose THC only when administered i.p. and using THC-E-gel; 2) THC induced analgesia only when administered i.p. and using THC-E-gel, though i.p. administration is more sensitive; and 3) THC reduces core body temperature only when administered i.p. and using THC-E-gel, though i.p. administration is more sensitive.

Next, we analyzed the cannabimimetic responses of individual mice following THC (10 mg/15 ml) E-gel access and how the CB_1R inverse agonist, SR1, administered 1h into the consumption window influences these responses (**Figure 3e**). Cannabimimetic responses increased linearly as a function of increasing amount of THC consumed, demonstrating a significant relationship between the amount of THC consumed and cannabimimetic readouts (**Figure 3f-h**). Confirming the involvement of CB_1R, SR1 blocked the three THC-induced cannabimimetic responses (**Figure 3f-h**). These results indicate that consumption of THC-E-gel evokes robust CB_1R-dependent cannabimimetic behavioral responses in adult mice that are comparable to *i.p.*-THC administration when measuring hypolocomotion, and less potent when compared to *i.p.*-THC administration when measuring analgesia and reduction in core body temperature.

Consumption of high-dose THC-E-gel results in concomitant increases in the levels of THC and its metabolites in brain tissue.

The PK profile of THC (5 mg/kg) administered by *i.p.* results in peak circulating concentrations of THC, its bioactive metabolite, 11-OH-THC, and its inactive metabolite, 11-COOH-THC, in brain after 2 h that reach approximately: 1000 pmol/g of THC, 300 pmol/g of 11-OH-THC and 100 pmol/g of 11-COOH-THC (29). To determine the PK profile of high-dose THC-E-gel consumption (10 mg) and considering the hypolocomotion behavior occurring during the consumption window, we collected plasma and brain tissue samples after 1 h of consumption, at the end of the 2 h access period, as well as 30 min (2.5 h) and 24 h (26 h) following the 2 h access period (**Figure 4a**). **Figure 4b** shows THC levels in the brain reached 500-600 pmol/g tissue between 1h and post 2.5 h time-point and was below 50 pmol/g tissue after 24 h. Remarkably, 11-OH-THC and 11-COOH-THC levels in brain increased concomitantly to THC levels, reaching 400-500 pmol/g tissue and 200-350 pmol/g tissue, respectively, between 1h and the post 2.5 h time-point, and both were also below 50 pmol/g tissue after 24 h. Thus, levels of both CB_1R agonists THC and 11-OH-THC peaked after 1 h of high-dose THC-E-gel consumption, matching the hypolocomotion response measured at 40 min during the 2 h consumption period (**Figure 2**). Furthermore, THC and 11-OH-THC levels in brain tissue were drastically lower after 24 h but still detectable, as previously reported (29-31). THC levels in plasma reached approximately 400 pmol/g tissue at the 1 h time-point and decreased thereafter (**Figure 4c**). 11-OH-THC and 11-COOH-THC levels were lower at the 1 h time-point and similarly decreased thereafter. Note that 11-OH-THC and 11-COOH-THC levels peaked after 2 h of consumption which contrasts with the early onset hypolocomotive response measured in **Figure 2** after 40 min of gelatin access. Thus, PK analysis of high-dose THC-E-gel consumption demonstrates parallel accumulation of THC and 11-OH-THC in the brain, a

unique profile that is quite different than the previously established PK profile resulting from THC-*i.p.* injection (29, 32).

Modeling THC doses based on the cannabimimetic responses triggered by *i.p.* THC injections and THC-E-gel consumption.

To further establish the pharmacological relationship between *i.p.* THC injections and THC-E-gel consumption after 1 h and 2 h consumption along with the low variability in the cannabimimetic responses triggered by both routes of administrations, we calculated "predicted THC doses" by comparing their cannabimimetic responses across experiments (**Figure 5a**). Thus, we extrapolated the relative *i.p.* dose for each cannabimimetic response triggered by consumption by plotting the cannabimimetic response following consumption onto the dose-response curve of THC-*i.p.* as reference (**Figure 5b-d**). **Figure 5b-d** also shows that 1 h access to high-dose THC-E-gel triggered greater cannabimimetic responses compared to 2 h access. Consequently, this resulted in a higher "predicted *i.p.* dose" shown by dotted lines tracked to the *i.p.* dose-response curves. Of note, 1 h access to high-dose THC-E-gel triggered stronger hypolocomotion and reduction in core body temperature corresponding to 10.3 and 11.6 mg/kg THC *i.p.*, respectively, and analgesia corresponding to 4.5 mg/kg THC *i.p.* (**Figure 5b-d**). By contrast, 2 h access to high-dose THC-E-gel triggered a comparable response in the three cannabimimetic behaviors corresponding to 3-4 mg/kg THC *i.p.* (**Figure 5b-d**). **Figure 5e** illustrates the predictive value of these calculations, and the larger variability for the 1 h access predicted dose of 8.81 ± 2.19 mg/kg *i.p.* and 3.69 ± 0.25 mg/kg *i.p.* for 2 h access, a 2.4-fold higher predicted dose after 1 h access. The variability between the cannabimimetic response for the 1 h access results suggest that a difference in the PK profile of THC at 1 h compared to 2 h access (see **Figure 4**). Together, these results indicate that consumption of high-dose

THC-E-gel triggers strong cannabimimetic responses, comparable to *i.p.* injections of THC between 4-12 mg/kg.

Differences between sex-dependent acoustic startle responses in response to *i.p.* injection of THC and high-dose THC-E-gel consumption.

Acoustic startle responses in mice are a well-established preclinical approach to evaluate an unconditional reflex characterized by the rapid contraction of muscles to a sudden and intense startling stimulus. It is an especially useful measure in preclinical research as it is consistent across species and involves simple neural circuitry in sensorimotor gating (33). It is known that *i.p.* injection of THC (6 and 10 mg/kg) reduces acoustic startle (17, 25, 34). However, whether startle response is affected in a sex-dependent manner or is altered by lower dose THC is unknown. Thus, we next sought to extend studies on the effect of *i.p.* injection of THC on acute acoustic startle in male and female mice and compare these results to high-dose THC-E-gel consumption. We measured the acute startle responses as the peak velocity (Vmax as measured by an accelerometer) following audible tones either 1 h after *i.p.* administration of THC (from 0.1 to 10 mg/kg) or immediately after access to high-dose THC-E-gel, and immediately after treatment measured the startle response to increasing tone power (80, 90, 100, 105, 110, and 120 dB) (**Figure 6a**). We found an overall more pronounced startle response in males than in females, as well as a biphasic response to increasing doses of THC in males but not females (**Figure 6b-c)**. For example, 1 mg/kg THC increased the startle response to 120 dB by 2-fold in males and had no effect in females, and 10 mg/kg THC reduced the startle response to 120 dB by 4.3-fold in males and by 3-fold in females (**Figure 6d**). THC-E-gel consumption by males and females triggered a remarkably different startle

response, here characterized by only males exhibiting an increase in response when allowing access for 2 h to THC-E-gel, leading to a 2.2-fold increase in the startle response to 120 dB (**Figure 6e-f**). These results indicate that THC administered *i.p.* triggers an inverted U-shape impairment of acoustic startle responses that is more pronounced in males than in females; and that only males exhibit an increased acoustic startle response when exposed for 2 h to high dose THC-E-gel. Using the male and female-specific dose-responses in **Figure 6d**, and the predicted *i.p.* dose described in **Figure 5e**, an acoustic startle response (Vmax) to a 120 dB tone following 10 mg THC-E-gel consumption was predicted as: in cm/min, 389.9 (1 h) and 708.8 (2 h) for females, and 900.7 (1 h) and 1854.3 (2 h) for males. **Figure 6g-h** compares the acoustic startle response to a 120 dB tone of male and female mice and the *i.p.* predicted response after 1 h and 2 h access to high-dose THC. Predicted startle responses in males exposed to high-dose THC-E-gel for both 1 and 2 h access was within the standard error of the measured startle response to a 120 dB tone, whereas only the 1 h predicted startle response in females was within the standard error of the measured response (**Figure 6g-h**). This prediction of THC-impaired psychotomimetic behaviors after a temporally modified access paradigm further illustrates the robust nature of our new THC-E-gel consumption model as an effective behavioral paradigm for investigating voluntary high-dose THC consumption.

Discussion

Here we report a novel experimental behavioral model that enables one to examine the behavioral impact of voluntary oral consumption of high-dose THC by adult mice. Daily access to E-gel for 2 h over a two-day period incentivizes robust consumption, and at the highest dose tested here (10 mg), mice of both sexes consumed ~30 mg/kg THC in 2 h on the second day. Acute consumption of THC triggers commonly established cannabimimetic responses, the

potencies of which were right shifted compared to the responses measured with *i.p.* injections. Furthermore, we discovered that acute consumption of high-dose THC-E-gel increases the acoustic startle response in males to a greater extent than it does in females; and that *i.p.* injection of THC triggers a dose-dependent, inverse U-shaped, impairment of acoustic startle response that was also more pronounced in males than females. Our study provides important translational results at two levels: acute consumption of THC by rodents and its impact on acoustic startle response, a preclinical measure of psychotomimetic responses.

Mice of both sexes consumed similar amounts of VEH-CTR-gel and VEH-E-gel, and none consumed more than 20% of their daily caloric intake indicating comparable consumption behaviors. However, consumption of high-dose THC-CTR-gel (4 mg) was inconsistent, and 41% of the mice completely avoided consumption (as assessed by an unbroken gelatin surface at the end of the 2 h access period) (**Figure 1c**). By contrast, consumption of high-dose THC-E-gel (10 mg, i.e., 2.5X more concentrated) was consistent with a total consumption rate of 0.95g/2 h (**Figure 1d**). This difference in consumption between THC-CTR-gel (4 mg) and THC-E-gel (10 mg) is likely due to the chocolate flavoring in Ensure™ that masks the strong odor and bitter taste of high-dose THC and its aversive properties. Significantly, mice that consumed the high-dose THC-E-gel on Day 2 consumed remarkably less VEH-E-gel on Day 3 and increased their latency to start consuming the control mixture (Supplementary Figure S3c). This is potentially due to the development of aversive conditioned associations to high-dose THC associated with the E-gel. Thus, the novel experimental model reported here also enables the study of aversive memory to high-dose THC during voluntary oral consumption.

I.p. injection of THC induces hypolocomotion, analgesia, and hypothermia in mice with different median effective doses (ID_{50}, 1.3, 3.9 and 14.4 mg/kg, respectively) (**Figure 3b-d**). By comparison, 1 h access to high-dose THC-E-gel produced cannabimimetic responses that paralleled the ID_{50} of *i.p.* injections and are equivalent to an *i.p.* dose of ~9 mg/kg. Also, 1 h access to high-dose THC-E-gel evoked a more pronounced cannabimimetic response compared to 2 h access, agreeing with prior studies which have shown that oral gavage increases brain peak concentration of THC 1-2 h after administration (35). Oral consumption also increases 11-OH-THC levels in the brain with comparable kinetics and concentration as THC, and the levels of both cannabinoids decrease in parallel (**Figure 4b-c**). Considering that ~600 pmol/g of THC and 11-OH-THC is roughly equivalent to 3 nM of both compounds in the brain that persists over several hours, and both activate CB_1R with comparable potencies, our results suggest that the accumulation of both THC and 11-OH-THC in the brain might contribute to cannabimimetic responses (36). Finally, considering that voluntary oral consumption of high-dose THC results in nanomolar concentrations of THC and 11-OH-THC that continuously activate CB_1R for several hours, the time-dependent reduction in cannabimimetic response that follows their maximal response may be due either to CB_1R desensitization/tolerance or to redistribution of the drug within brain parenchyma away from its biophase.

An *i.p.* injection of THC 6 and 10 mg/kg in male mice reduces acoustic startle behaviors (25, 34). We show here that THC-*i.p.* induces a dose-dependent biphasic behavioral response that is more pronounced in males than females, demonstrating sex-dependent sensorimotor behaviors, and confirming that THC impacts neurocognitive function in a sex-dependent manner (Supplementary Figure S4) (37-39). High-dose THC-E-gel consumption also increased

the response to acoustic startle preferentially in males compared to females. Whether the dose of THC formulated in E-gel can be increased to levels that remain palatable to mice and might trigger the pronounced reduced acoustic startle measured with high dose THC injection *i.p.* remains an open question. Analysis of the behavioral responses following *i.p.* injection and consumption of THC-E-gel enabled us to propose a model that correlates the doses of THC capable of producing comparable behavioral responses. The flexibility of the THC-E-gel experimental approach may extend its utility as a substitute for traditional *i.p.* injections, bridging the translational gap between preclinical investigations and human use. For example, the THC-E-gel experimental model can be easily modified and implemented to measure, in a less invasive manner, additional mouse behaviors including self-administration and preference/aversion, paradigms that require multiple treatment regimens.

In conclusion, our study outlines a new experimental model that achieves robust voluntary oral consumption of THC in adult mice by formulating THC in a chocolate-flavored sweetened E-gel. Given the recent rise in edible cannabis products containing high-dose THC (40), the model allows for the relevant and important translational investigation of psychotomimetic responses in mice that voluntarily consume the drug.

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Figure Legends:

Figure 1: E-gel promotes heightened voluntary oral consumption of THC by adult mice.

a) Mice were given free access to vehicle (VEH), or THC formulated in either CTR-gel or E-gel for 2 h on Day 1 and Day 2. **b**) Consumption was determined by weighing gelatin at the end of each session. **c**) Consumption of CTR-gel on Day 2 is decreased after addition of THC. **d**). Consumption of E-gel on Day 2 is maintained after addition of THC. **e**). Dose of THC consumed, in mg/kg, when formulated in either CTR-gel or E-gel on Day 2. Results are mean ± S.E.M. Consumption compared by T-test to respective VEH *p<0.05, **p<0.01, and ***p<0.001.

Figure 2: High-dose THC reduces locomotion during the exposure period

a) Over a 3-day exposure paradigm, mice received 3 days of E-gel with either VEH or THC (10mg/15ml) E-gel on day 2. Gelatin cup weights were measured, and animal activity recorded throughout consumption window. **b-d**) Cumulative gelatin consumption recorded every 10 minutes throughout the 2 h exposure window over the 3-day paradigm. VEH (black) and THC (purple) groups received access to VEH on day 1 (**b**), VEH or THC on day 2 (**c**), and VEH on day 3 (**d**). **e**) Total gelatin consumption after 2 h of access to gelatin was plotted comparing VEH and THC treatment groups. Significant decreases in THC group consumption were recorded by paired T-test on days 2 and 3 compared to VEH group (***p<0.001). f) Animal consummatory and locomotor behavior was tracked during gelatin exposure window. **g**) Distance traveled recorded every 5 min over the 3-day paradigm, similar as to **b-d**. **h**) Total distance traveled (cm) after 2 h of gelatin access was plotted comparing VEH and THC groups, significant decrease in locomotion recorded on day 2 by paired T-test (**p<0.01).

Figure 3: THC-E-gel consumption triggers CB_1R-dependent behaviors

a) Diagram of behavioral paradigm after *i.p.* or gelatin administration. b-d) Dose-dependent behavioral responses for hypolocomotion (**b**), analgesia (**c**), and hypothermia (**d**) after THC exposure. Administration by *i.p.* (grey) is plotted on x-axis by single bolus injection while CTR-gel (green) and E-gel (purple) are plotted based on average THC consumed after 2 h exposure window. **e**) Diagram of THC-E-gel exposure, behavioral measurements, and SR1 injection (by *i.p.*) at 1 h. **f-h**) Individual behavioral responses for hypolocomotion (**f**), analgesia (**g**), and

hypothermia (**h**) for each animal. Individual points are plotted based on individual THC consumption with a linear regression to show correlation between consumed THC and behavioral output (linear regression p-values: f=0.003, g<0.001, h<0.001). SR1 treated mice are plotted (red) based on consumed THC after exposure to 10 mg/15 ml THC-E-gel with a linear regression to show no correlation across three behaviors (linear regression p-values: f=0.09, g=0.44, h=0.45).

Figure 4: Consumption of high-dose THC-E-gel results in concomitant increases in the levels of THC and its metabolites in brain tissue

a) Diagram outlining gelatin exposure paradigm where blood and brain samples were collected immediately following 1 h and at 2, 2.5, and 26 hours from the beginning of 2 h access to 10mg/15ml THC-E-gel. **b**) Brain concentration of THC, 11-OH-THC, and COOH-THC after E-gel exposure, 1 h access is separated due to a reduced total access time to THC-E-gel compared to the other time points. **c**) Plasma concentrations for the three compounds plotted similarly to **b**.

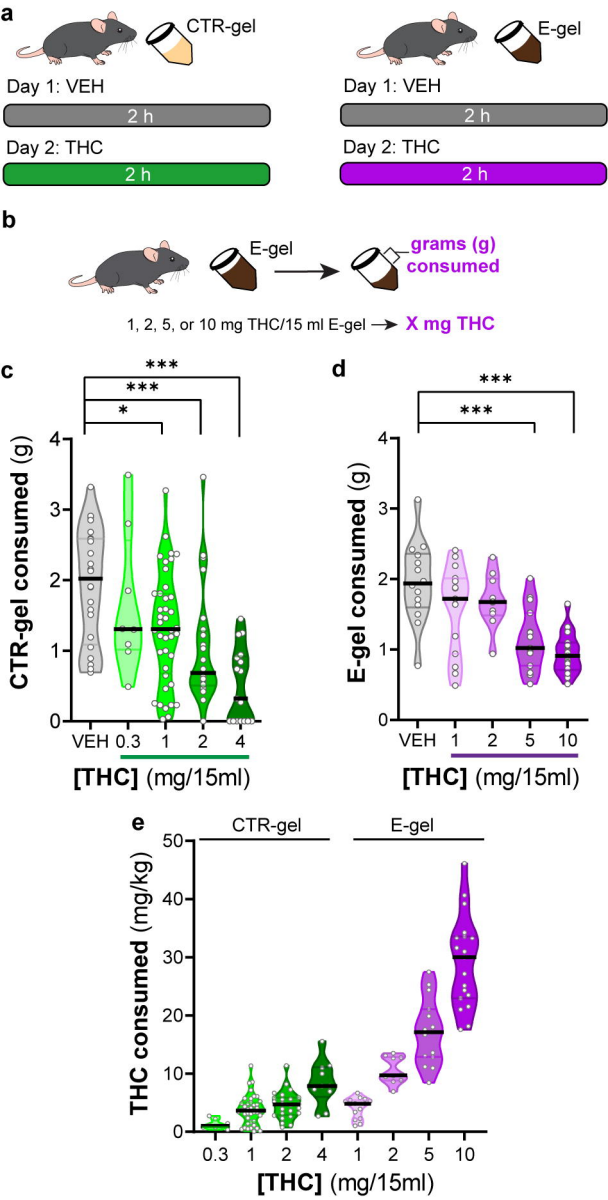
Figure 5: Modeling THC doses based on the cannabimimetic responses triggered by *i.p.* THC injections and THC-E-gel consumption

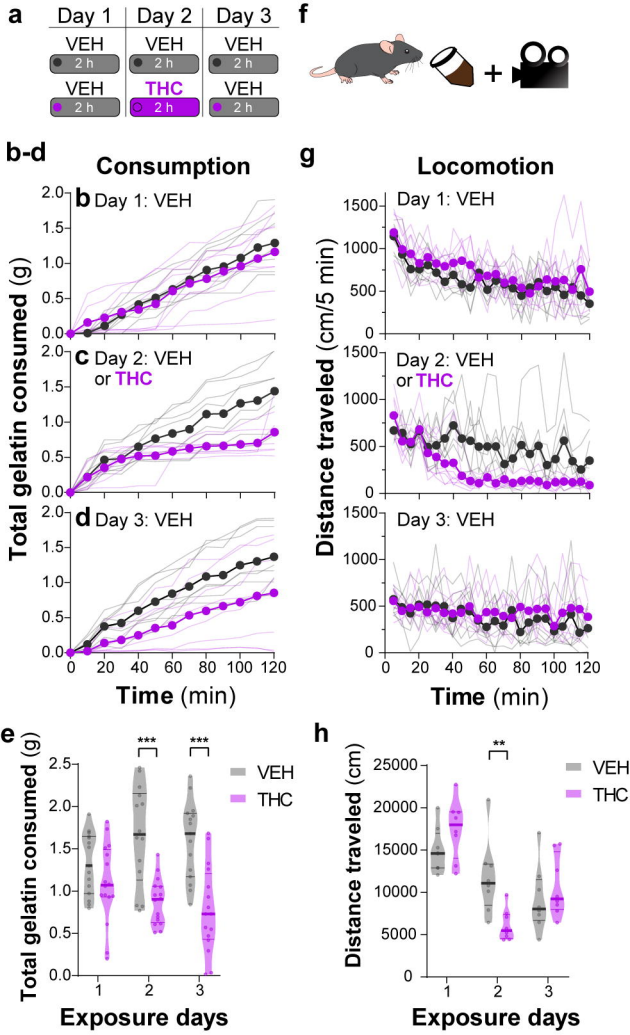
a) Diagram of 1 h and 2 h THC-E-gel exposure and *i.p.* administration with behavioral tests. **b-d**) Cannabimimetic responses after THC administration by *i.p.* and subsequent dose-response curve in grey. Responses after 1 h or 2 h exposure to 10 mg THC-E-gel are plotted with dotted lines tracking to relative THC-*i.p.* dose response. **e**) Predicted *i.p.* dose after 1 h and 2 h THC-E-gel exposure window from all three triad behaviors.

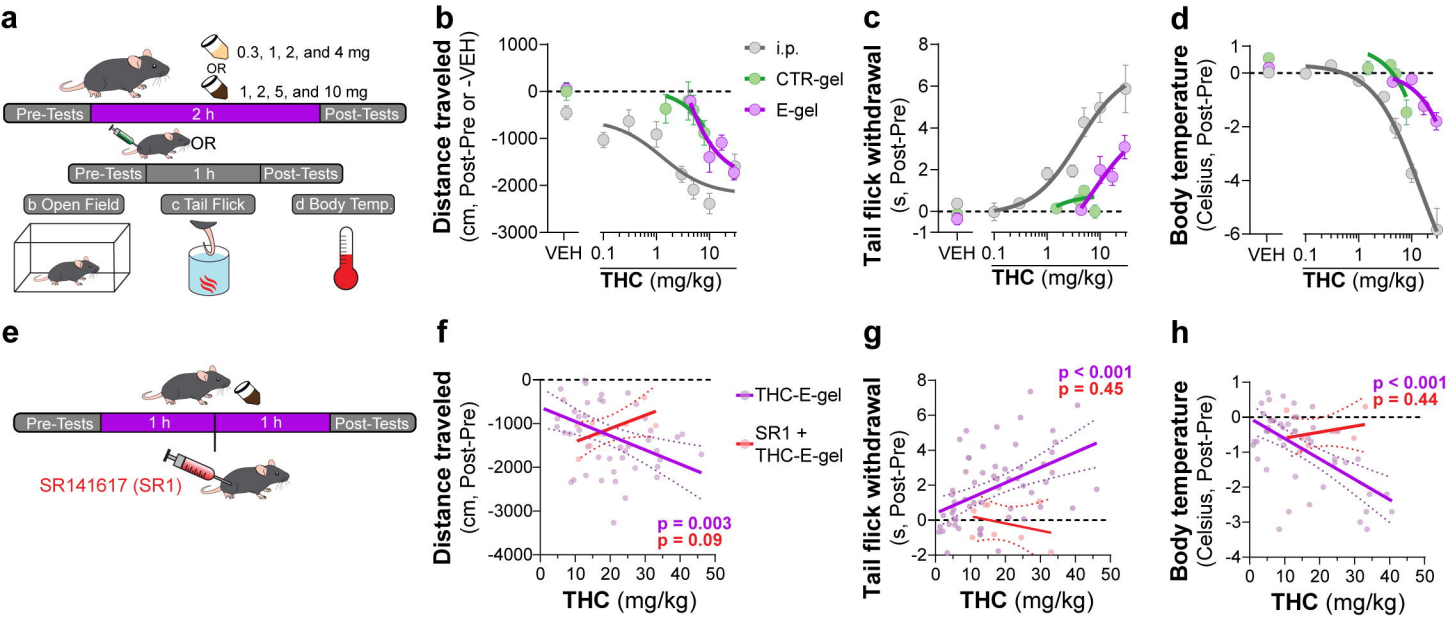
Figure 6: Differences between sex-dependent acoustic startle responses in response to *i.p.* injection of THC and high-dose THC-E-gel consumption

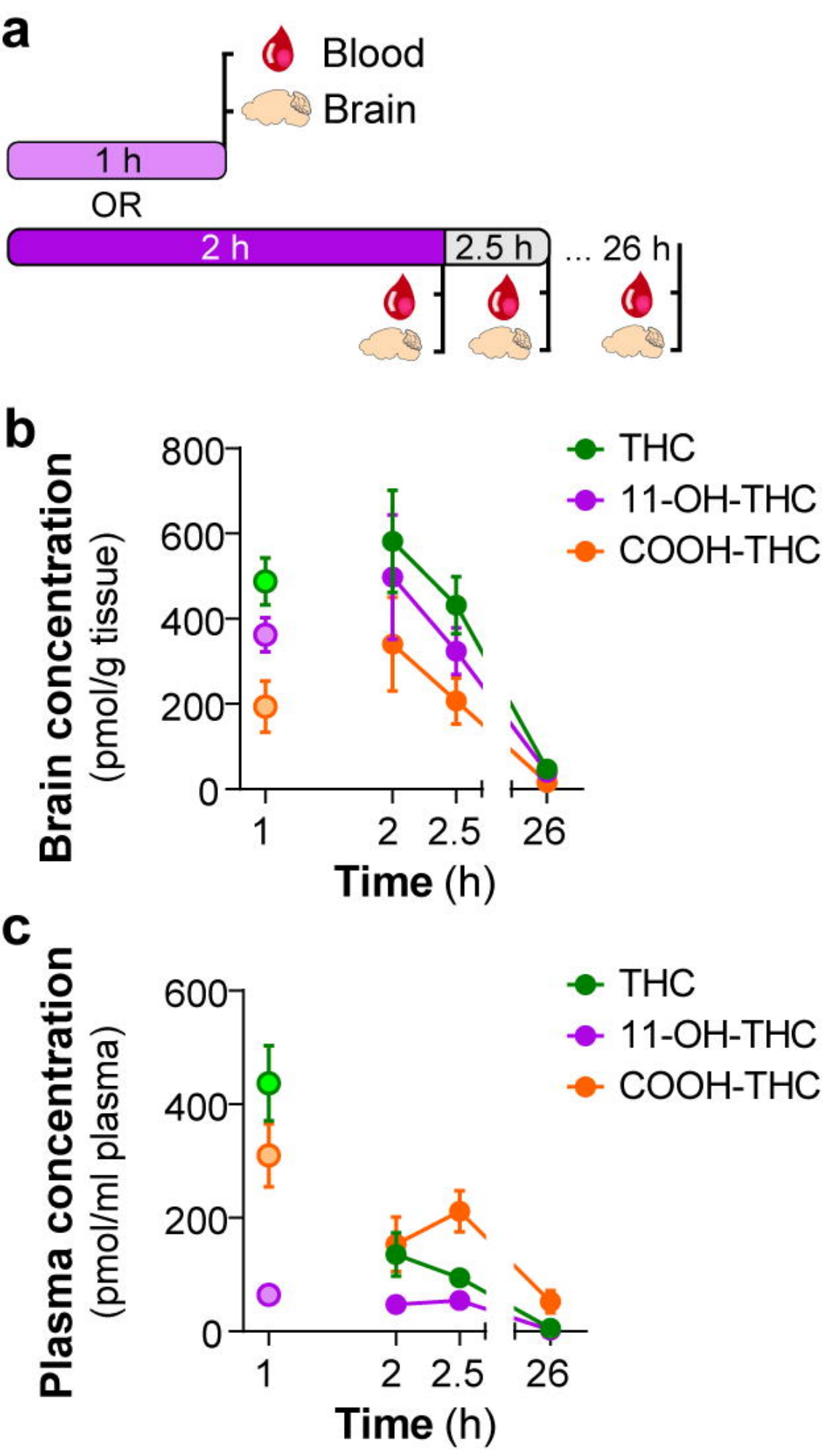
a) Diagram of THC-E-gel exposure or *i.p.* administration followed by acoustic startle response behavioral testing. **b-c**) Male and female acoustic startle responses after *i.p.* administration of THC in response to escalating tones (80, 90, 100, 105, 110, and 120dB) following *i.p.* administration of THC in males (**b**) and females (**c**). **d**) Male and female acoustic startle dose-responses to a 120dB tone after *i.p.* THC administration. Results are mean ± S.E.M. One-way ANOVA comparing VEH and *i.p.* THC dose between males and females **p<0.01, and

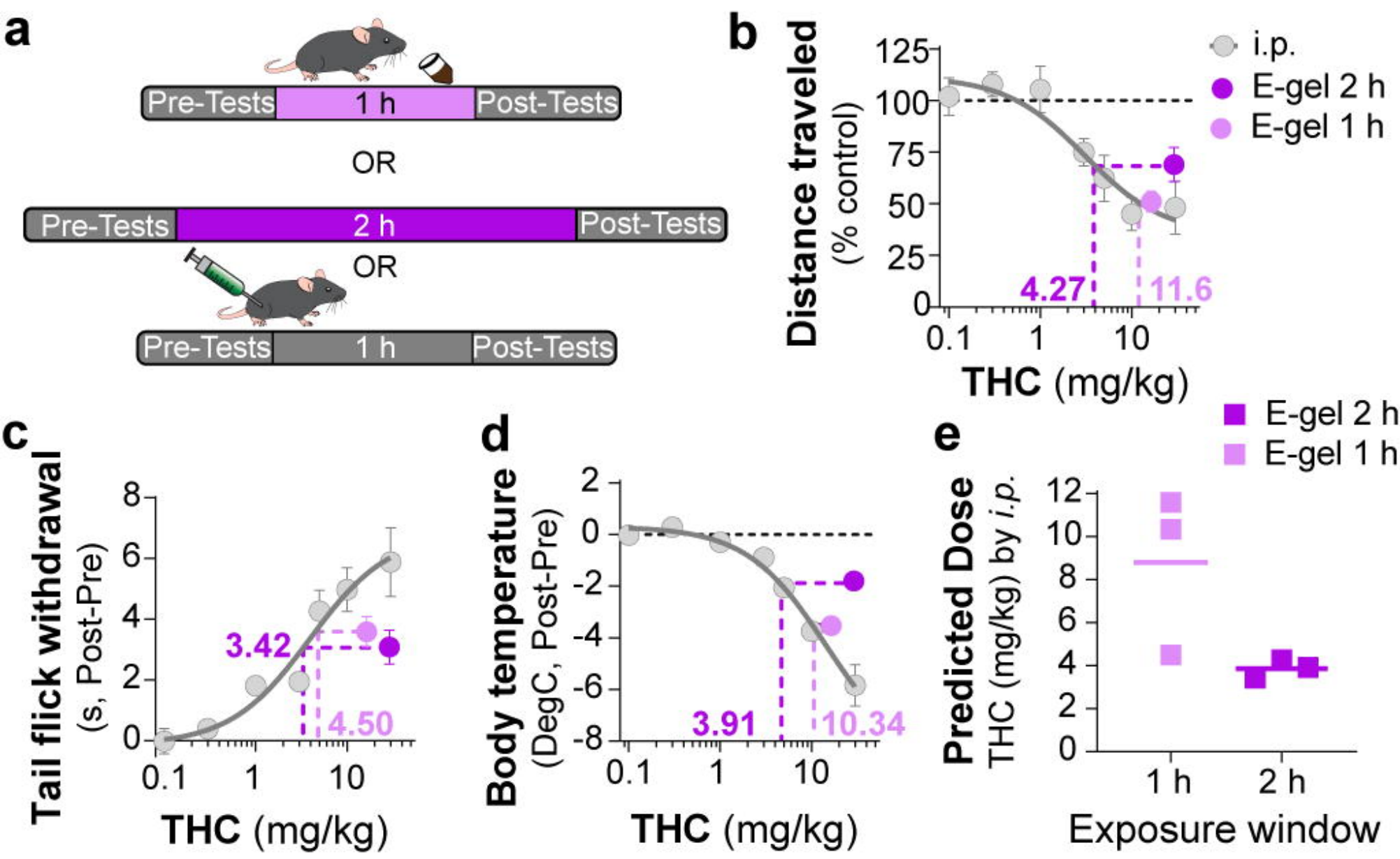
***p<0.001. **e-f**) Male and female acoustic startle responses after 1 h or 2 h THC E-gel exposure in response to escalating tones (80, 90, 100, 105, 110, and 120dB. **g-h**) Startle response to a 120dB tone for males (**g**) and females (**h**) after 1 h or 2 h access to THC E-gel. Predicted doses calculated from a second order polynomial of *i.p.* dose responses are plotted to show the consistency in predicted dose response after E-gel exposure.

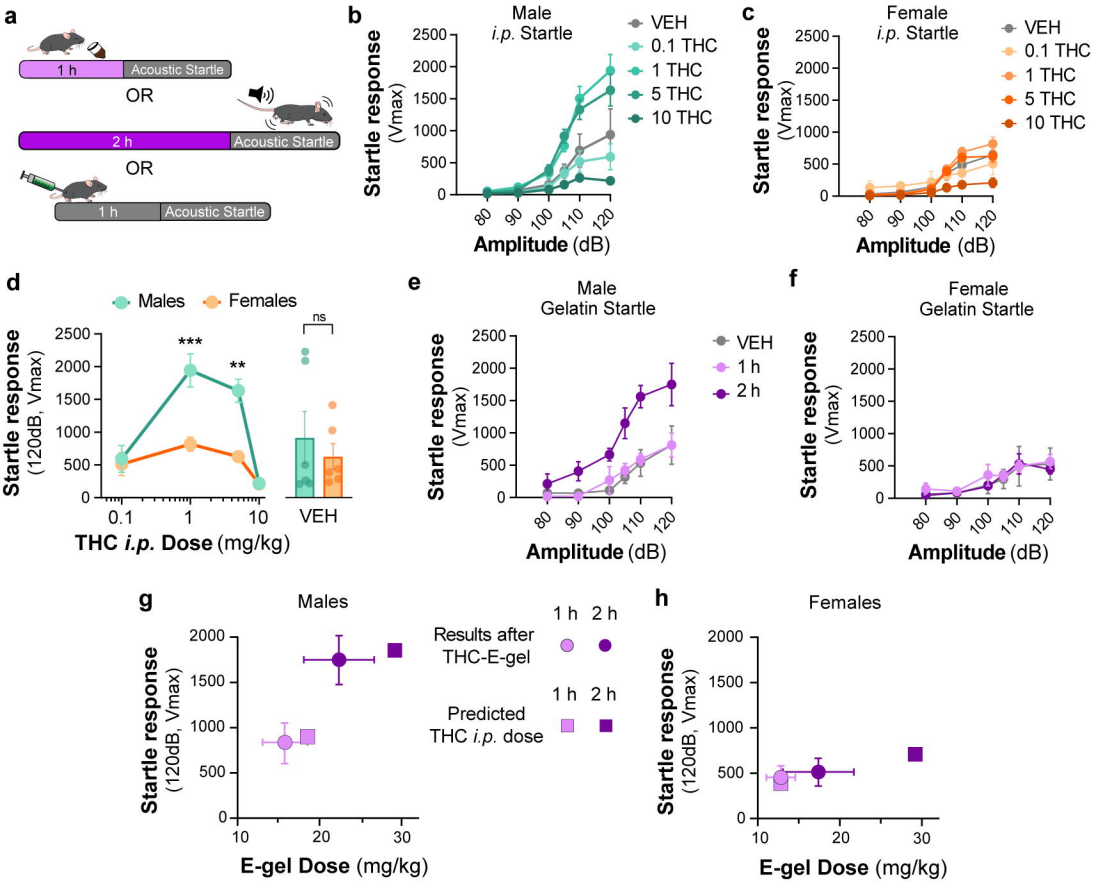

a
CTR-gel
Day 1: VEH
2 h
Day 2: THC
2 h
E-gel
Day 1: VEH
2 h
Day 2: THC
2 h
b
E-gel
grams (g) consumed
1, 2, 5, or 10 mg THC/15 ml E-gel → X mg THC
c
CTR-gel consumed (g)
***
***
*
VEH 0.3 1 2 4
[THC] (mg/15ml)
d
E-gel consumed (g)
***
***
VEH 1 2 5 10
[THC] (mg/15ml)
e
CTR-gel
E-gel
THC consumed (mg/kg)
0.3 1 2 4 1 2 5 10
[THC] (mg/15ml)


a
Day 1
Day 2
Day 3
VEH
2 h
THC
f
b-d
Consumption
b Day 1: VEH
c Day 2: VEH or THC
d Day 3: VEH
Total gelatin consumed (g)
Time (min)
g
Locomotion
Day 1: VEH
Day 2: VEH or THC
Day 3: VEH
Distance traveled (cm/5 min)
Time (min)
e
Total gelatin consumed (g)
***
VEH
THC
Exposure days
h
Distance traveled (cm)
**
Exposure days


a
0.3, 1, 2, and 4 mg
OR
1, 2, 5, and 10 mg
Pre-Tests
2 h
Post-Tests
OR
Pre-Tests
1 h
Post-Tests
b Open Field
c Tail Flick
d Body Temp.
b
Distance traveled
(cm, Post-Pre or -VEH)
i.p.
CTR-gel
E-gel
VEH
THC (mg/kg)
c
Tail flick withdrawal
(s, Post-Pre)
d
Body temperature
(Celsius, Post-Pre)
e
Pre-Tests
1 h
1 h
Post-Tests
SR141617 (SR1)
f
Distance traveled
(cm, Post-Pre)
THC-E-gel
SR1 +
THC-E-gel
p = 0.003
p = 0.09
g
Tail flick withdrawal
(s, Post-Pre)
p < 0.001
p = 0.45
h
Body temperature
(Celsius, Post-Pre)
p < 0.001
p = 0.44


a
Blood
Brain
1 h
OR
2 h
2.5 h
... 26 h
b
Brain concentration
(pmol/g tissue)
800
600
400
200
0
1
2
2.5
26
Time (h)
THC
11-OH-THC
COOH-THC
c
Plasma concentration
(pmol/ml plasma)
600
400
200
0
1
2
2.5
26
Time (h)
THC
11-OH-THC
COOH-THC


a
Pre-Tests
1 h
Post-Tests
OR
Pre-Tests
2 h
Post-Tests
OR
Pre-Tests
1 h
Post-Tests
b
Distance traveled
(% control)
125
100
75
50
25
0
4.27
11.6
0.1
1
10
THC (mg/kg)
i.p.
E-gel 2 h
E-gel 1 h
c
Tail flick withdrawal
(s, Post-Pre)
8
6
4
2
0
3.42
4.50
0.1
1
10
THC (mg/kg)
d
Body temperature
(DegC, Post-Pre)
2
0
-2
-4
-6
-8
3.91
10.34
0.1
1
10
THC (mg/kg)
e
E-gel 2 h
E-gel 1 h
Predicted Dose
THC (mg/kg) by i.p.
12
10
8
6
4
2
0
1 h
2 h
Exposure window


a
1 h
Acoustic Startle
OR
2 h
Acoustic Startle
OR
1 h
Acoustic Startle
b
Male
i.p. Startle
VEH
0.1 THC
1 THC
5 THC
10 THC
Startle response
(Vmax)
Amplitude (dB)
c
Female
i.p. Startle
d
Males
Females
***
**
ns
Startle response
(120dB, Vmax)
THC i.p. Dose (mg/kg)
VEH
e
Male
Gelatin Startle
1 h
2 h
f
Female
Gelatin Startle
g
Males
Results after
THC-E-gel
Predicted
THC i.p. dose
E-gel Dose (mg/kg)
h
Females