Effects of acute Δ9 -tetrahydrocannabinol on behavior and the endocannabinoid system in HIV-1 Tat transgenic female and male mice

Barkha J. Yadav-Samudrala
University of North Carolina at Chapel Hill
Ben L. Gorman
University of North Carolina at Chapel Hill
Hailey Dodson
University of North Carolina at Chapel Hill
Shreya Ramineni
University of North Carolina at Chapel Hill
Diane Wallace
University of North Carolina at Chapel Hill
Michelle R. Peace
Virginia Commonwealth University
Justin L. Poklis
Virginia Commonwealth University
Wei Jiang
Medical University of South Carolina
Sylvia Fitting (sfitting@email.unc.edu)
University of North Carolina at Chapel Hill

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Abstract

Cannabis use is highly prevalent especially among people living with HIV (PLWH). Activation of the anti-inflammatory and neuroprotective endocannabinoid system by phytocannabinoids, i.e. Δ^9-tetrahydrocannabinol (THC), has been proposed to reduce HIV symptoms. However, THC's effects on HIV-associated cognitive impairments are unclear. Using HIV-1 Tat transgenic mice, the current study investigates acute THC effects on various behavioral outcomes and the endocannabinoid system.

Minor or no effects of THC doses (1, 3, 10 mg/kg) were noted for body mass, body temperature, locomotor activity, and coordination, but spontaneous nociception was significantly decreased, with Tat induction increasing antinociceptive THC effects. Anxiogenic effects of THC (10 mg/kg) were demonstrated in Tat(−) females and males compared to vehicle-treated mice, with overall increased anxiety-like behavior in females compared to males. Object recognition memory was diminished by acute THC (10 mg/kg) injections in Tat(−) but not Tat(+) females, without affecting males. For the endocannabinoid system and related lipids, no effects were noted for acute THC, but female sex and Tat induction was associated with elevated 2-AG, AEA, AA, CB_1R, CB_2R, FAAH and/or MAGL expression in various CNS regions. Further, females demonstrated higher AEA levels compared to males in most CNS structures, and AEA levels in the prefrontal cortex of Tat(+) females were negatively associated with recognition memory.

Overall, findings indicate that acute THC exposure exerts differential effects on behavior in the context of neuroHIV dependent on sex, potentially due to an altered endocannabinoid system, which may be of relevance in view of potential cannabis-based treatment options for PLWH.

1. Introduction

The use of cannabis is highly prevalent in people living with HIV (PLWH), with cannabis use being reported to be 2–3 times higher than use among the general US population (Whitfield et al., 1997; Cristiani et al., 2004; Pacek et al., 2018; Ellis et al., 2021). Besides recreational use, PLWH report using cannabis to alleviate HIV-related symptoms, such as pain, anxiety, stress, nausea, and loss of appetite (Woolridge et al., 2005; Shiau et al., 2017; Costiniuk et al., 2019; Wardell et al., 2022). Another important HIV complication is the development of mild-to-severe neurocognitive impairments, known as HIV-1-associated neurocognitive disorders (HAND). HAND affects 30–50% of PLWH despite effective antiretroviral therapy (ART) (Dore et al., 1999; Sacktor et al., 2002; Antinori et al., 2007; Heaton et al., 2011; Kesby et al., 2016). These complications are associated with HIV-induced persistent inflammation and immune activation (Gartner, 2000; Kaul et al., 2001; Harezlak et al., 2011). Activation of the anti-inflammatory and neuroprotective endocannabinoid system by phytocannabinoids, such as Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD), has been proposed to reduce some of these HIV symptoms (Ellis et al., 2020; Ellis et al., 2021; Watson et al., 2021; Basova et al., 2022; Yin et al., 2022). Nevertheless, the exact role and limits of cannabis use on cognition in the context of HIV are not clear.

Acute and chronic cannabis use in the general US population has been shown to profoundly impair attention, learning and memory, and various other domains of cognitive function (Fletcher et al., 1996; Solowij et al., 2002; Lundqvist, 2005; Crean et al., 2011; Bossong et al., 2014; Broyd et al., 2016; Curran et al., 2016; Busardo et al., 2017; Campbell et al., 2018; Morgan et al., 2018; Kasten et al., 2019; Curran et al., 2020; Kroon et al., 2021; Urits et al., 2021), as well as lead to structural and functional changes in the central nervous system (CNS) (Battistella et al., 2014; Broyd et al., 2016; Szutorisz and Hurd, 2018; Zehra et al., 2018). THC is the principal psychoactive component of cannabis and acts as a partial agonist at the cannabinoid type 1 and type 2 receptors (CB_1R and CB_2R, respectively) (Pertwee, 2008). It is known that THC produces the majority of its CNS-related behavioral effects through the activation of CB_1Rs (Wiley et al., 1995), whereas THC's action on the CB_2R is predominantly related to THC's anti-inflammatory effects in models of inflammation (Turcotte et al., 2016).

Whereas not much is known about the acute effects of cannabis in PLWH, research findings of chronic cannabis effects on neurocognition and the CNS in PLWH are divided. Whereas previous studies show no effects or negative effects of cannabis use in PLWH (Cristiani et al., 2004; Chang et al., 2006; Thames et al., 2016; Thames et al., 2017; Skalski et al., 2018; Watson et al., 2021), including exacerbating the existing learning and memory deficits seen in PLWH (Cristiani et al., 2004; Thames et al., 2016; Skalski et al., 2018), recent published literature finds trends toward enhanced learning and working-memory performance in PLWH cannabis users versus PLWH non-users (Watson et al., 2020; Basova et al., 2022). Similarly, whereas brain neuronal networks in PLWH non-users are altered compared to control HIV negative non-users, cannabis use in PLWH may return brain networks to control levels (Meade et al., 2019; Christopher-Hayes et al., 2021; Hall et al., 2021; Flannery et al., 2022). Variability of findings might be due to various factors, including disease progression (Cristiani et al., 2004; Chang et al., 2006), the onset and amount of cannabis use (Bonn-Miller et al., 2014; Thames et al., 2016; Thames et al., 2017; Skalski et al., 2018), and the cognitive domain assessed (Thames et al., 2016; Watson et al., 2020). Further, due to the wide variance in cannabis composition (Vergara et al., 2017; Cash et al., 2020), clarity is lacking concerning which cannabis components are responsible for their observed interactive effects with HIV and cognition. Lastly, as sex differences are present in HAND vulnerability (Failde-Garrido et al., 2008; Rubin et al., 2020; Dreyer et al., 2021), HIV Tat effects (Hahn et al., 2015; Jacobs et al., 2019; Zhao et al., 2020; Xu et al., 2022), human cannabis use (Cuttler et al., 2016; Matheson et al., 2020), cannabinoid effects (Wakley et al., 2014b; Wakley et al., 2014a; Cuttler et al., 2016; LaFleur et al., 2018; Sholler et al., 2021; Wiley et al., 2021; Henderson-Redmond et al., 2022), THC metabolism (Cuttler et al., 2016; Ruiz et al., 2021; Sholler et al., 2021; Arkell et al., 2022), cannabinoid receptor expression (Wakley et al., 2014b; Spindle et al., 2021) and activation (Farquhar et al., 2019), it is similarly vital to investigate effects of sex in this context.

Thus, in the present study we make use of the HIV-1 Tat transgenic mouse model to investigate the acute effects of THC (1, 3, 10 mg/kg) on various behavioral outcomes, including pain sensitivity, motor activity, motor coordination, anxiety, and object recognition memory. Further, effects of acute THC on the endocannabinoid system in the CNS are assessed, including the prefrontal cortex, striatum, cerebellum,

and spinal cord, with endocannabinoid levels and related lipids being quantified via mass spectrometry and expression of cannabinoid receptors and endocannabinoid degradative enzymes being assessed by Western blot analyses.

2. Materials And Methods

2.1. Animals

HIV-1 IIIB Tat_{1-86} transgenic female and male mice [~ 9–10 months of age, *N* = 38(20f)] were used in the present study and developed on a hybrid C57BL/6J background such that Tat induction was brain-restricted and inducible by doxycycline (DOX), as previously described (Chauhan et al., 2003; Bruce-Keller et al., 2008). Briefly, mice expressing reverse tetracycline transactivator driven by a glial fibrillary acidic protein promoter (*GFAP-rtTA*) were crossed with transgenic mice expressing the *TRE-tat* gene or lacking the *tat* transgenic gene. Progeny were genotyped by PCR (Transnetyx, Inc., Cordova, TN) at 4 weeks of age to differentiate those expressing the Tat protein [transgenic Tat(+) mice] versus control mice not expressing the Tat protein [control Tat(−) mice]. Tat expression in Tat(+) mice was induced 1 month prior to the start of experiments and maintained throughout the study timeline by an *ad libitum* DOX chow diet (6 mg/g; product TD.09282, Envigo, NJ, USA). Control Tat(−) mice received the same DOX chow diet to evaluate any non-specific actions of DOX ingestion. Animals were group housed (2–4 mice per cage) on a reversed 12 h light/dark cycle (lights off at 8:00 AM) and had free access to water and chow. Experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85 – 23). All procedures were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee (IACUC).

2.2. Drugs

Δ^9-tetrahydrocannabinol (THC; #12068, Cayman Chemical, Ann Arbor, MI) was dissolved in a vehicle solution consisting of a mixture of ethanol, Kolliphor® EL (Sigma-Aldrich, #C5135, St. Louis, MO), and saline (0.9% NaCL), in a 1:1:18 ratio. Administration of vehicle and THC doses (1, 3, or 10 mg/kg) were given via subcutaneous (s.c.) injections at a volume of 10 µL/g of body mass. Drug treatments were randomized in all experiments.

2.3. Experimental design

For four days, mice were trained in the rotarod task (3 trials per day) and habituated to the chambers in which locomotor activity was assessed. THC doses including vehicle (0, 1, 3, 10 mg/kg) were then administered on four subsequent days distributed in a Latin-square design. To ensure clearance of THC doses, experiments were conducted with at least 48 h between each testing day. Locomotor activity, body temperature and spontaneous nociception, and rotarod trials were conducted 30 min, 45 min, and 60 min post-injection, respectively. Body weight was taken 24 h after drug injections to monitor animals' health. The novel object recognition (NOR) and elevated plus maze (EPM) tasks were performed 4 and 6 days later, respectively, and vehicle or 10 mg/kg THC injections were administered ~ 30 min prior to these

tasks. Drug injections, physiological assessments (body mass and body temperature), and behavioral measures were typically conducted between 9 am and 1 pm each day.

2.4. Behavioral procedure

2.4.1. Body mass and body temperature. Animals were weighed to monitor animals' health. Mice were gently picked up and placed in a transparent container located on a dedicated and portable scale balance (Fisher Scientific, #S94793A, Waltham, MA). After body mass was recorded, mice were returned to their home cage. Body temperature was taken using a non-contact infrared thermometer (Fisher Scientific, #12-894-005) prior to tail-flick and hot-plate assays as previously described (Kawakami et al., 2018). Briefly, each mouse was picked up by its tail and the abdomen was exposed by letting the mouse hold on to the lip of an open cage with its forepaw. In cases where hind paws were resting on the surface of the thermometer it was ensured that feet were not obstructing the sensor from the abdominal surface. Temperature measurements were taken when mice were relatively less mobile to ensure the same location of the body was measured each time.

2.4.2. Tail-flick and hot-plate assays. The tail-flick and hot-plate assays were used to assess spontaneous heat-evoked nociception with focusing on supraspinal and spinal pathways, respectively (Singh et al., 2018).

For the tail-flick test, the distal 1/3 of the tail of each mouse was placed in a water bath (Thermo Scientific, Precision General-Purpose Water Bath, Model 181, Waltham, MA) with the temperature maintained at 56 ± 0.1°C. The latency to remove the tail from the bath was recorded as a measure of nociception. A maximum of 10 s was used as a cut-off latency to prevent tissue damage.

Immediately following the tail-flick assay the hot-plate test was conducted. Mice were placed on the surface of a hot plate (55 ± 0.1°C; IITC, Inc., MOD 39, Woodland Hills, CA) within a Plexiglas™ chamber (15 cm height, 10 cm diameter) to avoid escape. Mice were removed immediately after withdrawing or licking a paw or jumping and total time was recorded as a measure of nociception. A maximum of 15 s was used as a cut-off latency to prevent tissue damage.

2.4.3. Locomotor activity. The locomotor activity task was used to assess unprompted locomotion in mice. Mice were placed in standard mouse experimental chambers (MED Associates, ENV-307W; 22 cm x 18 cm floor, Fairfax, VT) that were housed in sound and light attenuating cubicles (MED Associates, ENV-022MD). Testing occurred in a dark room illuminated by red fluorescent lighting with white noise from an air conditioning unit located inside the room. The testing room was kept at 22°C room temperature with 30% humidity. Locomotor activity was recorded for 10 min (600 s) using night vision cameras (Amcrest, FullHD 1080P 2MP Dome, Houston, TX) mounted to the ceiling of the light and sound attenuating chambers, with videos recorded to a Security Recorder (Amcrest, AMDV8M16-H5). The floor of the chamber was divided into four quadrants of equal size and shape, and the overall locomotor activity of mice was quantified by the number of times their nose crossed from one quadrant to another. Numerical data were generated from the 10 min videos of the trials by a team of trained experimenters blinded to

treatment condition. Each video was independently processed by two experiments with an overall interrater reliability (Cronbach's α) of α = 0.963. The locomotor activity data (# of crossings) represent the average score between each of the two experimenters.

2.4.4. Rotarod. The accelerating rotarod test was used to assess motor function and coordination, as described previously (Jones and Roberts, 1968). Mice were placed on a rotarod apparatus consisting of a raised, rubber-covered rod (30 mm diameter, elevated 18 cm) divided into five sections (each 50 mm wide) to allow for simultaneous testing of multiple mice Harvard Apparatus, #76–0770, Holliston, MA). Mice were placed on the rod and allowed to habituate for 60 s prior trial start. At start the accelerating rod initially rotated at 2 rotations per minute (rpm) and increased speed by 1 rpm every 7 s up to 60 rpm, which was reached by 7 min (420 s). The amount of time (s) each animal could remain on the rotating rod without falling or looping was recorded.

2.4.5. Elevated plus maze (EPM). The EPM task is a common measure of anxiety-like behavior as increased apparent anxiety in mice is related to increased preference for darker, enclosed spaces (Shoji and Miyakawa, 2021). Mice were placed in the center of an elevated, plus-shaped apparatus (San Diego Instruments, #7001 – 0316, San Diego, CA) in which two arms were sheltered with opaque beige walls (15 cm tall, closed arms) and two arms were unsheltered (open arms). Each arm was 30 cm long, 5 cm wide, and 38 cm above the ground. The EPM was placed on a table and indirect lighting was used to ensure that all four arms were similarly illuminated with light levels being kept consistent in consecutive experimental animals and days. Mice were free to explore the apparatus for 10 min (600 s) and their exploration behavior was recorded with a video camera (GoPro, Hero 6 Black, v02.10, San Mateo, CA) mounted overhead. Numerical data were generated from videos of the trials by a team of 5 trained experimenters blinded to treatment condition. The number of nose pokes into the open arms and the time spent in the open arms [data are presented as percent time spent in the open arms from total exploration time (600 s)] was used as indices of open space-induced anxiety in mice. Each video was independently processed by two experiments with an overall interrater reliability of α = 0.992 for latency and α = 0.990 for number of pokes. For both dependent variables data represent the average score between each of the two experimenters.

2.4.6. Novel object recognition (NOR). The NOR task is a well-established measure of object recognition memory which relies upon the natural tendency of mice to explore new stimuli (Ennaceur, 2010; Lueptow, 2017). The NOR task took place in a hexagonal arena constructed of lightly textured high-density white polyethylene (50 cm wide, 23 cm tall, courtesy of G.F. League Co., Inc., Greenville, SC). The task consisted of three phases: habituation, training, and testing, as described previously (Lueptow, 2017). All phases were recorded with a video camera (GoPro Hero 6 Black, v02.10, San Mateo, CA) mounted overhead. During the habituation phase, mice were habituated to the testing apparatus by allowing mice to explore the arena for 5 min. In the training phase, which occurred 1–2 days later, two identical objects (familiar objects) were presented equidistant from the center and mice were left to explore the arena for 10 min before they were returned to their home cage for 2 h. After 2 h mice were injected (s.c.) with vehicle or THC (10 mg/kg) and kept in their home cage for another 30 min before the testing phase started. For the

testing phase, one of the familiar objects (randomized for each mouse) was replaced by a new object (novel object) and mice were allowed to explore the arena for 10 min to assess object recognition memory. The duration of time that mice spent exploring the objects (familiar and novel) during the testing session was recorded. Object exploration was defined as being directly adjacent to and facing the object at a distance closer than 1 cm. As mice show an innate preference for novelty, their preference for the novel object was used to quantify successful object recognition memory (Ennaceur, 2010). The glass objects used were equal in size (20 cm high, 7 cm wide) but differed in shape, with one object being a square prism and the other a curved cylinder. Both glass objects had no natural significance to mice. The use of each as the familiar or novel object was randomized for each mouse. Numerical data were generated from videos of the trials by a team of 5 trained experimenters blinded to treatment condition. Each video was independently processed by two experiments with an overall interrater reliability of $\alpha = 0.986$ for the novel object and $\alpha = 0.977$ for the familiar object. Data represent the average score between each of the two experimenters. Total exploration time (s) of both objects (familiar and novel) was calculated to assess object exploration time. Discrimination between the objects was calculated using a discrimination index, calculated as the difference in the time spent exploring the novel object minus the familiar object, divided by the total time spent exploring the objects (Lueptow, 2017; Miedel et al., 2017). A discrimination index of 0 indicates no preference, 1 indicates complete preference for the novel object, and − 1 indicates complete preference for the familiar object.

2.5. Analysis of endocannabinoids and related lipids

Endogenous cannabinoid ligands, including the two main endocannabinoids AEA and 2-AG, *two* peroxisome proliferator activator receptor (PPAR) ligands, including *N*-oleoyl ethanolamide (OEA) and *N*-palmitoyl ethanolamide (PEA), and arachidonic acid (AA), were quantified via ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) from four CNS regions (prefrontal cortex, striatum, cerebellum, and spinal cord) of the male and female Tat transgenic mice after completion of behavioral experiments. Mice received an acute s.c. injection of either vehicle or THC (10 mg/kg) 1 h prior sacrifice. Mice were sacrificed by rapid decapitation following isoflurane-induced anesthesia and the four CNS regions were dissected, collected, and snap-frozen in liquid nitrogen within 10 min after decapitation. Samples were stored at − 80°C until use. Samples from the right hemisphere were processed and substrates quantified in a similar manner to previous studies (Dempsey et al., 2019; League et al., 2021). Details on the extraction and quantification of endocannabinoids and related lipids can be found in the **Supplemental Methods**.

2.6. Western blot analysis

For the western blot analysis prefrontal cortex samples from the left hemisphere of Tat transgenic mice were quantified for cannabinoid receptor protein expression, including CB_1Rs and CB_2R, and endocannabinoid degradative enzymes, including fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). Samples were homogenized on ice in an appropriate volume of ice-cold Pierce™ RIPA lysis and extraction buffer (Thermo Scientific, Cat# 89900) with Halt™ phosphatase

(Thermo Scientific, Cat# 78420) and protease inhibitor cocktail (Thermo Scientific, Cat# 87786). Homogenized tissue lysates were centrifuged at 10,000 rcf for 10 min at 4°C. Pierce™ BCA protein assay kit (Thermo Scientific, Cat# 23227) was used to determine the protein concentration. Protein lysates were suspended in sample buffer containing NuPAGE™ LDS Sample Buffer (Invitrogen™, Cat# NP0007, Waltham, MA) and XT Reducing Agent (BioRad, Cat# 1610792, Hercules, CA) in 1:2.5 ratio and denatured at 85°C for 10 min. Equal amounts of protein (20 µg/lane) were resolved in 10% Bis-Tris Criterion™ XT Precast Gels and XT MOPS running buffer (BioRad, Cat# 1610799) at 120 volts for 1.5 h using Criterion™ vertical electrophoresis cell (BioRad, Cat# 1656001). Electrophoretic transfer of proteins from the gel to Immobilon®-P PVDF membranes (Millipore Sigma, Cat# IPVH00010, Burlington, MA) was carried out in 10x Tris/Glycine buffer (BioRad, Cat# 1610734) at 1–4°C and 100 volts for 1 h using Criterion™ blotter with wire electrodes (BioRad, Cat#1704071). Blots were rinsed with phosphate-buffered saline (PBS), incubated with Intercept® blocking buffer (LI-COR Biosciences, Cat# 727-70001, Lincoln, NE) at room temperature for 1 h. Blots were incubated with primary antibodies overnight at 4°C in Intercept® blocking buffer with 0.2% Tween-20. Primary antibodies used in this study were, anti-CB_1R (rabbit polyclonal; Proteintech, Cat# 17978-1-AP, 1:1000 dilution, Rosemont, IL), anti-CB_2R (rabbit polyclonal; AbClonal, Cat# A1762, 1:1000 dilution, Wobum, MA), anti-FAAH (mouse monoclonal; Abcam, Cat# ab54615, 1:1000 dilution, Cambridge, United Kingdom), and anti-MAGL (rabbit polyclonal; Abcam, Cat# ab24701, 1:1000 dilution). Anti- antibody (mouse monoclonal; Abcam, Cat# ab125247, 1:15,000 dilution) was used as a housekeeping protein. Following the primary antibody incubation, the blots were washed 3x with PBST (PBS with 0.1% Tween-20) and incubated with IRDye® 680RD Donkey anti-Mouse IgG (LI-COR Biosciences, Cat# 926-68072, 1:15,000 dilution) and IRDye® 800CW Donkey anti-Rabbit IgG (LI-COR Biosciences, Cat# 925-32213, 1:15,000 dilution) secondary antibodies at room temperature for 1 h in Intercept® blocking buffer with 0.2% Tween-20 and 0.01% SDS. The blots were washed 3x with PBST and bands were detected using Odyssey® CLx infrared imaging system (LI-COR Biosciences, USA) and analyzed in Empiria studio® software version 2.3.0 (LI-COR Biosciences, USA). All blots were normalized to the housekeeping protein GAPDH and fold-change was calculated using a control sample represented on all blots.

2.7. Analysis of Δ⁹-THC levels and its metabolites

A new cohort of drug-naive Tat(−) female and male mice ($n = 7$ per sex) was s.c. injected with an acute dose of 10 mg/kg THC to confirm Δ -THC levels and its metabolites, including 11-nor-9-carboxy-Δ -tetrahydrocannabinol (THC-COOH) and 11-hydroxy-Δ -tetrahydrocannabinol (THC-OH), in the plasma and mouse cortex. Mice were sacrificed 1 h after THC injections following isoflurane-induced anesthesia, and plasma and cortex samples were taken within 5 min. Plasma samples were prepared from fresh blood drawn via cardiac puncture (**Supplemental Methods**). Following cardiac puncture, cortex samples were dissected, collected, and snap-frozen in liquid nitrogen immediately after decapitation. Samples were stored at − 80°C until use.

Extraction and quantification of Δ -THC levels and its metabolites in plasma and cortex samples were conducted as follows. A 100 µL aliquot of plasma was extracted with 600 µL of 80:20 MeOH:H_2O.

Samples were shaken for 15 min then centrifuged for 10 min at 20,000 rcf. Supernatant was dried down and reconstituted with 100 µL of MeOH. Cortex tissue was extracted with 600 µL of 80:20 MeOH:H_2O and sonicated for 15 min to break up tissue and then centrifuged for 10 min at 20,000 rcf. Cortex extracts were dried down and reconstituted in 100 µL MeOH.

Samples were analyzed with a Waters Acquity Ultra-Performance Liquid Chromatography (UPLC) system tandem to a Thermo Scientific TSQ Vantage triple quadrupole mass spectrometer. Separations were conducted using a Waters BEH C18 150 mm x 2.1 mm column; water with 0.1% formic acid was used for mobile phase A and acetonitrile with 0.1% formic acid was mobile phase B. A flow rate of 0.25 mL/min was used with starting conditions of 65% A. A linear decrease was performed to 45% A over 2 minutes followed by a hold for 1 minute. Another decrease to 20% A at 7 min was performed followed by another to 5% A at 8 minutes. A sharp decrease was performed from 5 to 0% A over 2 min with a curve of 3 (curve of 6 is linear). There was a hold at 100% B for 3 min followed by a re-equilibration step from 13.5 to 16 min. An injection volume of 10 µL was utilized. Multiple reaction monitoring (MRM) was utilized to monitor specific transitions for Δ -THC, THC-COOH, and THC-OH (**Supplemental Table S1**).

2.8. Statistical analysis

All data are presented as mean ± the standard error of the mean (SEM). Data sets of animal's health, spontaneous nociception, and motor performance were analyzed by three-way mixed analysis of variances (ANOVAs) with drug dose (4 levels: vehicle, 1, 3, 10 mg/kg THC) as a within-subjects factor and sex (2 levels: females, males) and genotype [2 levels: Tat(−) mice, Tat(+) mice] as between-subjects factors. Data sets from the novel object recognition task and elevated plus maze were analyzed by three-way ANOVAs with drug (2 levels: vehicle, 10 mg/kg THC), sex (2 levels: females, males), and genotype [2 levels: Tat(−) mice, Tat(+) mice] as between-subjects factors. Data sets from THC levels and it's metabolites were analyzed by two-way mixed ANOVAs with sample (2 levels: plasma, cortex) as a within-subjects factor and sex (2 levels: females, males) as a between-subjects factor. To determine specific differences between groups for each sex, separate ANOVAs were conducted for females and males. ANOVAs were followed by Bonferroni's post hoc tests when appropriate. Data sets for endocannabinoids and related lipid molecules were analyzed for each lipid molecule by one-way repeated ANOVAs with CNS regions [4 levels: prefrontal cortex, striatum, cerebellum, spinal cord] as a within-subjects factor or multivariate three-way ANOVAs with CNS regions as the multivariate variable and drug (2 levels: vehicle, 10 mg/kg THC), sex (2 levels: females, males), and genotype [2 levels: Tat(−) mice, Tat(+) mice] as between-subjects factors. The relationship between selected behavioral and/or lipid molecules was assessed via correlation and simple regression analyses. An alpha level of $p < 0.05$ was considered significant for all statistical tests. SPSS Statistics 25 (IBM, Chicago, IL) and Prism GraphPad 8.0 (San Diego, CA) was used for data analysis and data graphing, respectively.

3. Results

3.1. Body mass and body temperature

Body mass (g) and body temperature (°C) were taken to ensure animal's health was not substantially affected by the study procedure (Fig. 1A**B**). Animals were weighed 24 h after acute administration of THC doses (0–10 mg/kg, Fig. 1A**)**. A three-way ANOVA with drug dose as a within-subjects factor and sex and genotype as between-subjects factors demonstrated a significant main effect of sex, $F(1, 34) = 6.6$, $p = 0.014$. Male mice (35.3 ± 0.92, $n = 18$) weighed more on average than females (31.9 ± 0.95, $n = 20$), which was not affected by genotype. No significant THC dose effects or interactions were noted.

Animal body temperature was also measured as an indicator of animal's health 45 min following acute THC injections (Fig. 1B). No significant main effect or interactions were noted, indicating that body temperature was not altered by THC doses, sex, or genotype. Overall, acute administration of THC doses did not lead to significant changes in body mass or body temperature, confirming the continued health of the animals after acute drug exposure.

3.2. Spontaneous heat-evoked nociception

The tail-flick and hot-plate assays were conducted 45 min after drug injections to assess effects of acute THC (0–10 mg/kg) exposure on heat-evoked pain like behaviors in the Tat transgenic mouse model (Fig. 1C**D**). The tail-flick test was used to assess spinal-related spontaneous nociception (Fig. 1C). A three-way ANOVA with drug dose as a within-subjects factor and sex and genotype as between-subjects factors demonstrated a significant main effect of THC doses, $F(1, 34) = 146.7$, $p < 0.001$. Bonferroni's post hoc tests indicated that all THC doses, including vehicle, significantly differed from each other ($p < 0.001$) except 1 mg/kg from 3 mg/kg, indicating that THC administration induced dose-dependent antinociception, thus increasing latency to remove the tail. Importantly, a significant main effect of genotype was noted, $F(1, 34) = 13.1$, $p < 0.001$, that was altered by drug dose, drug dose x genotype interaction: $F(1, 34) = 11.4$, $p < 0.001$, with Tat(+) mice showing higher response latencies, thus antinociception, with increasing THC doses. When conducting individual comparisons for each drug dose, significant genotype effects were noted for 3 mg/kg THC, $F(1, 34) = 5.7$, $p = 0.023$, and 10 mg/kg THC, $F(1, 34) = 13.5$, $p < 0.001$. No significant sex effect or interactions were noted.

The hot-plate test was used to assess supraspinal-related spontaneous nociception (Fig. 1D). Similar to the tail-flick data, a three-way ANOVA with drug dose as a within-subjects factor and sex and genotype as between-subjects factors demonstrated a significant main effect of THC doses, $F(1, 34) = 12.3$, $p < 0.001$, with elevated THC doses increasing time of paw withdrawal or lick, thus inducing antinociception. Bonferroni's post hoc tests indicated that the 10 mg/kg THC dose significantly differed from all other groups ($p < 0.001$). No other effect or interaction was significant.

Overall, spontaneous heat-evoked nociception is significantly decreased in a THC dose-dependent manner, thus withdrawal latencies are increased, and Tat induction enhances THC-induced antinociception in the spinal-related tail-flick task at higher THC doses.

3.3. Locomotor activity and rotarod coordination

To understand the effects of acute THC (0–10 mg/kg) exposure on motor function we assessed locomotor activity and rotarod performance 30 min and 1 h after injections, respectively (Fig. 1E**F**). The locomotor activity task was conducted to assess effects of acute THC administration in Tat transgenic mice on motor activity (Fig. 1E). A three-way ANOVA with drug dose as a within-subjects factor and sex and genotype as between-subjects factors demonstrated a significant main effect of THC doses, $F(1, 34) = 3.4$, $p = 0.021$, with Bonferroni's post hoc tests indicating higher activity after 1 mg/kg THC administration compared to vehicle exposure ($p < 0.001$). Specifically, female Tat(−) and female Tat(+) mice, but not males, demonstrated a significant drug dose effect [$F(1, 27) = 3.0$, $p = 0.047$ and $F(1, 27) = 3.4$, $p = 0.031$, respectively], with female Tat(−) mice showing higher activity after 1 mg/kg THC compared to vehicle administration ($p = 0.013$) and Tat(+) mice showing higher activity after 10 mg/kg THC compared to vehicle ($p = 0.012$). No other significant effects were noted.

The rotarod task was conducted to investigate effects of THC exposure in Tat transgenic mice on motor coordination and function (Fig. 1F). A three-way ANOVA with drug dose as a within-subjects factor and sex and genotype as between-subjects factors demonstrated no significant effects or interactions.

Overall, a slight increase of locomotor activity was noticed after administration of THC doses, specifically for female mice, but no effects were noted for motor coordination.

3.4. Anxiety-like behavior

The elevated plus maze task was conducted 30 min after injections to assess the effects of acute THC (0 and 10 mg/kg) exposure in Tat transgenic mice on anxiety-like behavior (Fig. 2). The dependent measures included percent time spent in open arms and number of pokes into open arms. A three-way ANOVA with drug, sex, and genotype as between-subjects factors was conducted for each measure. For percent time spent in open arms (Fig. 2A) a significant main effect of sex was noted, $F(1, 30) = 4.2$, $p = 0.050$, in which males showed less anxiety compared to females, thus spending more time in the open arms. No significant effects or interactions were noted for drug or genotype.

For number of pokes into open arms (Fig. 2A) a significant drug main effect was noted, $F(1, 30) = 5.4$, $p = 0.028$, in which THC decreased pokes into the open arms compared to vehicle exposure. Interestingly, this was specifically noted for Tat(−) females, $F(1, 8) = 6.3$, $p = 0.037$, and Tat(−) males, $F(1, 7) = 7.6$, $p = 0.028$, but not Tat(+) animals.

Overall, males showed less anxiety compared to females and THC administration appeared to increase anxiety-like behavior in Tat(−) animals but not Tat(+) mice.

3.5. Novel object recognition

The NOR task was conducted 30 min after injections to assess the effects of acute THC (0 and 10 mg/kg) exposure in Tat transgenic mice on novel object recognition memory (Fig. 3). The dependent measures included total time of exploring both the novel and familiar objects and time spent exploring the novel object over the familiar object (discrimination index). Due to the natural tendency to explore

new stimuli, the preference for a novel object means that presentation of the familiar object exists in animal's memory and thus presents successful object recognition memory (Ennaceur, 2010). For total exploration time (Fig. 3A), a three-way ANOVA including drug, sex, and genotype as between-subjects factors, demonstrated a significant main effect of drug, $F(1, 30) = 13.4$, $p < 0.001$, with THC administration decreasing object exploration time compared to vehicle exposure. This main effect of drug was specifically noted for Tat(−) females, $F(1, 8) = 15.6$, $p = 0.004$, and Tat(−) males, $F(1, 7) = 7.4$, $p = 0.030$, but not Tat(+) animals. Further, a significant main effect of sex was noted, $F(1, 30) = 4.2$, $p = 0.049$, with female mice showing higher exploration behavior compared to males. Lastly, a sex x genotype interaction was noted, $F(1, 30) = 10.70$, $p = 0.003$, with Tat(+) female mice demonstrating decreased object exploration time compared to Tat(−) females, whereas no effect was noted for males More specifically, vehicle exposed Tat(−) females significantly differed from vehicle exposed Tat(+) female, $F(1, 8) = 13.0$, $p = 0.007$.

For object recognition memory, the discrimination index was used, with complete preference for the novel object equal 1, no preference equal 0, and complete preference for the familiar object equal − 1 (Fig. 3B). A two-way ANOVA for each sex revealed a significant drug x genotype interaction for females, $F(1, 16) = 4.5$, $p = 0.049$, but not males. Specifically, THC administration significantly impacted object recognition memory in Tat(−) females, $F(1, 8) = 5.9$, $p = 0.041$, without affecting recognition memory in Tat(+) females. In the presence of THC exposure the natural tendency of Tat(−) females preferring to explore the novel object was reversed to exploring the familiar object over the novel object. No other effects or interactions were noted.

Overall, total object exploration time was significantly higher in females compared to males and altered by genotype; THC administration decreased object exploration time in males and females, specifically for the Tat(−) groups. For object recognition memory, THC administration had no effect on males but differentially affected recognition memory in female mice by reversing the natural tendency of Tat(−) females without affecting the recognition memory in Tat(+) females, which showed no preference.

3.6. CNS levels of endocannabinoids and related lipids

To assess the impact of acute THC (0 and 10 mg/kg) exposure on the endogenous cannabinoid system, changes in levels of 2-AG, AEA, PEA, OEA, and AA were assessed 60 min after injections in various CNS regions of Tat transgenic female and male mice ($n = 4–5$ per group), including the prefrontal cortex, striatum, cerebellum, and spinal cord (**Supplemental Table S2**, Fig. 4). Lipid molecule concentrations (nmol/g) differed significantly between CNS region; 2-AG, $F(3, 111) = 41.7$, $p < 0.001$, demonstrated differences in expression levels between all CNS regions (p's < 0.001) with lowest 2-AG levels for the prefrontal cortex, followed by the striatum, cerebellum, and the highest 2-AG levels expressed in the spinal cord. AEA levels, $F(3, 111) = 23.7$, $p < 0.001$, were found to be higher in the PFC and striatum compared to the cerebellum and spinal cord (p's < 0.001). AA, $F(3, 111) = 17.6$, $p < 0.001$ and OEA, $F(3, 111) = 26.1$, $p < 0.001$, showed highest expression in the striatum compared to the other three CNS regions (p's < 0.01) and PEA levels, $F(3, 111) = 9.6$, $p < 0.001$, were also highest in the striatum but only differed significantly from the cerebellum and spinal cord (p's < 0.01, **Supplemental Table S2**, Fig. 4). To assess treatment

effects a multivariate analysis was conducted for each lipid molecule with drug, sex, and genotype and as between-subjects factors. No effects or interactions were noted for acute THC administration on any measure indicating that acute exposure of THC did not alter the endocannabinoid system and related lipid molecules. The most prominent findings were noted for AEA and AA levels with some minor effects for 2-AG (Fig. 4) and PEA and OEA (**Supplemental Table S2**).

For the prefrontal cortex (Fig. 4A), significant sex effects were noted for 2-AG, $F(1, 30) = 9.3$, $p = 0.005$, and for AEA, $F(1, 30) = 22.3$, $p < 0.001$, with females demonstrating higher 2-AG and AEA levels compared to male mice. For AA, a genotype effect was noted for the prefrontal cortex, $F(1, 30) = 6.1$, $p = 0.019$, that was altered by sex, $F(1, 30) = 7.5$, $p = 0.010$, with Tat(+) females showing higher AA levels compared to Tat(−) females, $F(1, 18) = 12.1$, $p = 0.003$, but no difference was noted for males. Specifically, for females this effect was noted for vehicle-exposed females, $F(1, 8) = 6.0$, $p = 0.040$, as well as THC-exposed females, $F(1, 8) = 5.5$, $p = 0.047$.

For the striatum (Fig. 4B), significant sex effects were noted for AEA, $F(1, 30) = 41.1$, $p < 0.001$, and AA, $F(1, 30) = 20.4$, $p < 0.001$, with females demonstrating higher AEA and AA levels compared to male mice. Further, a sex x genotype interaction was noted for AEA in the striatum, $F(1, 30) = 11.6$, $p = 0.002$, in which Tat(+) females showed higher AEA levels compared to Tat(−) females, $F(1, 18) = 7.7$, $p = 0.012$, but the reversed was true for males, $F(1, 16) = 5.5$, $p = 0.032$. Specifically, vehicle-exposed Tat(+) females showed higher AEA levels compared to vehicle-exposed Tat(−) females, $F(1, 8) = 5.8$, $p = 0.042$, without any other group showing significance.

For the cerebellum (Fig. 4C), a significant sex effect was noted for AA, $F(1, 30) = 11.3$, $p = 0.002$, with females demonstrating higher AA levels compared to male mice. No other significant effects or interactions were noted.

For the spinal cord (Fig. 4D), significant sex effects were noted for AEA, $F(1, 30) = 6.6$, $p = 0.015$, and AA, $F(1, 30) = 26.6$, $p < 0.001$, with females demonstrating higher AEA but lower AA levels compared to male mice. Further, AEA levels were significantly altered by genotype in the spinal cord, $F(1, 30) = 4.9$, $p = 0.034$, with upregulated AEA expression for Tat(+) mice compared to Tat(−) mice, with individually testing genotype effect for each sex not showing significance. In contrast, the sex x genotype interaction for AA levels in the spinal cord, $F(1, 30) = 6.1$, $p = 0.020$, showed lower AA levels in Tat(+) females compared to Tat(−) females, $F(1, 18) = 11.2$, $p = 0.004$, but no difference was noted for males. Specifically, for females this effect was noted for THC-exposed females, $F(1, 8) = 6.6$, $p = 0.033$, but not vehicle-exposed females. Lastly, for 2-AG, a significant genotype effect was found for the spinal cord, $F(1, 30) = 4.8$, $p = 0.036$, with higher 2-AG expression levels in Tat(+) mice compared to Tat(−) mice. Specifically, THC-exposed Tat(+) females showed higher 2-AG levels compared to THC-exposed Tat(−) females, $F(1, 8) = 7.0$, $p = 0.029$, without any other group showing significance. No other significant effects or interactions were noted.

Results for PEA and OEA mirrored a few of the findings for AEA, specifically some of the noted sex effects in the prefrontal cortex, even though opposite sex effects were noted for the striatum and spinal cord (**Supplemental Table S2**).

Overall, acute THC administration did not alter endocannabinoids or related lipid molecules in any CNS region. Interestingly, female mice showed higher AEA and/or AA expression levels in almost all CNS regions compared to males, except for AA levels in the spinal cord, with 2-AG being upregulated for females in the prefrontal cortex only. Further, Tat induction seemed to affect 2-AG, AEA and AA levels differently for males and females in some CNS regions, including the striatum, prefrontal cortex, and/or spinal cord.

3.7. Relationships between AEA, AA, and object recognition memory

As the most prominent findings were noted for AEA and AA levels, we assessed the relationship between levels of AEA and AA within each of the four CNS regions. Pearson correlations were conducted between both lipid molecules separately for females and males within each CNS region (Table 1). Data indicate that specifically in the prefrontal cortex significant positive relationships were noted with low AA levels being associated with low AEA levels and high AA levels being associated with high AEA levels. Note that correlations for AA and AEA across CNS regions revealed only selected significant relations (**Supplemental Results**). For the prefrontal cortex, significant correlations were further assessed by simple regression analyses. Results indicate predictability of AEA levels by AA accounting for 44% − 73% of total variance in the data. Specifically, for females AA predicted 44% of total variance in AEA data, $F(1, 18) = 14.07$, $p = 0.001$, which was carried by Tat(+) females only [61%, $F(1, 8) = 12.34$, $p = 0.008$]. For males, the AEA variance accounted for by AA was 46%, $F(1, 16) = 13.34$, $p = 0.002$, which was found to be significant for both genotypes [Tat(−) males: 73%, $F(1, 7) = 18.79$, $p = 0.003$; Tat(+) males: 64%, $F(1, 7) = 12.22$, $p = 0.010$].

Table 1
Pearson correlation matrix between AEA and AA levels within each of the four CNS regions separate for females and males. As 10 mg/kg THC administration had no effects on any of the assessed lipid molecules drug groups were combined.[a]

CNS region	Sex	Geno-type	Pearson Correlations within each CNS region: AEA vs. AA		
			r	*p*	*n*
Prefrontal cortex	Female	Tat(−)	-0.224	0.534	10
		Tat(+)	**0.779**	**0.008**	10
		(−) & (+)	**0.662**	**0.001**	20
	Male	Tat(−)	**0.854**	**0.003**	9
		Tat(+)	**0.797**	**0.010**	9
		(−) & (+)	**0.674**	**0.002**	18
Striatum	Female	Tat(−)	0.474	0.166	10
		Tat(+)	0.324	0.362	10
		(−) & (+)	0.427	0.060	20
	Male	Tat(−)	0.353	0.352	9
		Tat(+)	0.086	0.826	9
		(−) & (+)	0.336	0.172	18
Cerebellum	Female	Tat(−)	**0.634**	**0.049**	10
		Tat(+)	-0.431	0.213	10
		(−) & (+)	0.283	0.227	20
	Male	Tat(−)	0.154	0.692	9
		Tat(+)	-0.179	0.644	9
		(−) & (+)	-0.039	0.879	18
Spinal cord	Female	Tat(−)	-0.510	0.132	10
		Tat(+)	-0.395	0.259	10
		(−) & (+)	**-0.504**	**0.023**	20
	Male	Tat(−)	0.322	0.398	9

[a] No effects or interactions were noted for acute THC administration on any measure and are thus not shown in this table. Bolded values denote significant differences at $p \leq 0.05$. CNS, central nervous system; *n*, sample size.

CNS region	Sex	Geno-type	Pearson Correlations within each CNS region: AEA vs. AA		
			r	*p*	*n*
		Tat(+)	0.395	0.293	9
		(−) & (+)	0.431	0.074	18

[a] No effects or interactions were noted for acute THC administration on any measure and are thus not shown in this table. Bolded values denote significant differences at $p \leq 0.05$. CNS, central nervous system; *n*, sample size.

Next, we were interested in exploring the relationship between the two lipid molecules in the prefrontal cortex and the observed behavioral outcome in the novel object recognition task. Analyses were conducted separately for sex and genotype as both factors differentially affected object recognition memory and/or AEA and AA levels. As no effects were noted for males, only female correlation and simple regression data are shown (Fig. 5). Pearson correlations revealed a significant negative relationship between AEA levels and object recognition memory for Tat(+) females (exposed to vehicle and THC combined), with low AEA levels being associated with better object recognition memory, thus the preference to explore the novel object over the familiar object (Fig. 5A). A simple linear regression demonstrated predictability of object recognition memory (discrimination index) by AEA levels in the prefrontal cortex of Tat(+) females accounting for 44% of total variance in the data ($F(1, 8) = 6.31$, $p = 0.036$). No significant effect was noted for female Tat(−) mice (Fig. 5A). No significant effects were noted for AA levels and object recognition memory (Fig. 5B).

3.8. Expression levels of cannabinoid receptors and endocannabinoid degradative enzymes in the prefrontal cortex

To assess the impact of acute THC (0 and 10 mg/kg) exposure on cannabinoid receptors and cannabinoid catabolic enzymes, changes in protein expression levels of CB_1R, CB_2R, MAGL, and FAAH were assessed 60 min after injections in the prefrontal cortex of Tat transgenic female and male mice ($n = 4–5$ per group; Fig. 6). Data were normalized to the housekeeping protein GAPDH and fold-change was calculated using a control sample represented on all blots. Note that Pearson correlation analyses for object recognition memory and expression levels of CB_1R, CB_2R, MAGL, or FAAH revealed no significant relations (**Supplemental Table S3**). For CB_1R protein expression (Fig. 6A), a three-way ANOVA including drug, sex, and genotype as between-subjects factors, demonstrated a significant main effect of genotype, $F(1, 30) = 8.8$, $p = 0.006$, with Tat induction increasing CB_1R expression levels. Interestingly, the genotype effect was altered by sex with a significant sex x genotype interaction, $F(1, 30) = 26.1$, $p < 0.001$, with Tat(+) female mice demonstrating increased CB_1R expression compared to Tat(−) females, $F(1, 16) = 24.9$, $p < 0.001$, whereas no effect was noted for males. The upregulation of CB_1R expression in Tat(+)

female mice compared to Tat(−) females was note din the absence and presence of THC exposure ($F(1, 8) = 15.2$, $p = 0.005$ and $F(1, 8) = 10.2$, $p = 0.013$, respectively).

For CB_2R protein expression (Fig. 6B), a three-way ANOVA demonstrated a significant main effect of sex, $F(1, 30) = 4.0$, $p = 0.054$, with females showing higher CB_2R expression levels compared to males. No other significant effects were noted.

For MAGL enzyme expression (Fig. 6C), a three-way ANOVA demonstrated a similar effect as noted for CB_2R expression levels, with a significant main effect of sex, $F(1, 30) = 4.9$, $p = 0.035$, with females showing higher MAGL enzyme protein expression levels compared to males. No other significant effects were noted.

For FAAH enzyme expression (Fig. 6D), a three-way ANOVA demonstrated a significant main effect of sex, $F(1, 30) = 9.8$, $p = 0.004$, with females showing higher FAAH enzyme protein expression levels compared to males. Further, a significant main effect of genotype, $F(1, 30) = 25.3$, $p < 0.001$, with Tat induction increasing FAAH levels. This genotype effect was found in female mice, $F(1, 16) = 11.2$, $p = 0.004$, and male mice, $F(1, 14) = 16.4$, $p = 0.001$, specifically in the THC-treated groups (THC-exposed females: $F(1, 8) = 7.9$, $p = 0.023$; THC-exposed males: $F(1, 8) = 14.1$, $p = 0.006$) but not vehicle-exposed mice.

Overall, females showed higher expression levels in the prefrontal cortex for CB_2Rs, MAGL, and FAAH compared to males. Further, Tat induction increased CB_1R expression in Tat(+) female mice compared to Tat(−) females, as well as FAAH levels in THC-exposed Tat(+) females and THC-exposed Tat(+) male mice compared to their THC-exposed Tat(−) counterparts.

3.9. Plasma and cortex levels of THC and its metabolites

Levels of THC and its metabolites were assessed 60 min after acute THC (10 mg/kg) exposure using a different cohort of animals. Female and male Tat(−) mice ($n = 7$ per group) were subcutaneously injected with 10 mg/kg THC and sacrificed 1 h later, around the time when behavior was assessed (Fig. 7). THC levels and its metabolites, THC-COOH and THC-OH, were detected in all plasma (ng/mL) and cortex (ng/mg) samples. A two-way mixed ANOVA was conducted with sample as a within-subjects factor and sex as a between-subjects factor. For THC levels (Fig. 7A), a significant sample effect was noted, $F(1, 12) = 18.33$, $p < 0.001$, with higher concentrations in plasma compared to cortex samples. This effect was noted for females, $F(1, 6) = 75.32$, $p < 0.001$, and males, $F(1, 6) = 11.84$, $p = 0.014$. No sex effect or interaction was found. For THC-COOH levels (Fig. 7B), a significant sample effect was noted, $F(1, 12) = 95.28$, $p < 0.001$, with higher concentrations in plasma compared to cortex samples. This effect was found to be present in females, $F(1, 6) = 67.77$, $p < 0.001$, and males, $F(1, 6) = 28.38$, $p = 0.002$. Further, a significant main effect of sex was noted, $F(1, 12) = 9.84$, $p = 0.009$, that was altered by sample, sample x sex interaction: $F(1, 12) = 9.91$, $p = 0.008$, with higher THC-COOH levels found for female mice in plasma compared to males, $F(1, 12) = 9.87$, $p = 0.009$, but no differences was noted for cortex samples. For THC-OH levels (Fig. 7C), a significant sample effect was noted, $F(1, 12) = 50.70$, $p < 0.001$, with higher

concentrations in plasma compared to cortex samples. This effect was noted for females, $F(1, 6) = 16.89$, $p = 0.006$, and males, $F(1, 6) = 5.97$, $p = 0.050$. No sex effect or interaction was found.

Overall, levels of THC and its metabolites were detected in plasma and cortex after 1 h acute 10 mg/kg THC administration and found to be higher concentrated in plasma compared to cortex samples. THC levels did not differ based on sex, but its metabolite THC-COOH demonstrated a selected sex effect in which females had higher THC-COOH plasma levels compared to male mice.

4. Discussion

The present study investigated acute effects of Δ^9-THC in female and male Tat transgenic mice on various behavioral measures and the endocannabinoid system. Behavioral assessments demonstrated that Tat induction significantly altered acute effects of THC in multiple measures, including (1) enhancing THC's antinociceptive effects in the heat-evoked tail-flick task, (2) decreasing the anxiogenic effects of acute THC in female and male mice, and (3) diminishing THC's effects on object recognition memory in female mice without affecting males. Acute THC had no effects on the endocannabinoid systems and related lipids but female sex and Tat induction increased 2-AG, AEA, AA, CB_1R, CB_2R, MAGL, and/or FAAH expression in various brain regions.

Cannabinoids have been widely reported to be antinociceptive in animal models, often with distinct sex differences (Wakley et al., 2014a; LaFleur et al., 2018; Henderson-Redmond et al., 2022). In the present study, acute THC at various doses (1, 3, and/or 10 mg/kg) were found to have profound antinociceptive effects independent of sex. Importantly in the context of Tat induction, THC's antinociceptive effects were enhanced in the spinal-related tail-flick task at higher THC doses. This finding is interesting as opposite effects have been reported for opioid analgesic treatments in the context of HIV-1 infection (Koeppe et al., 2010; Palma et al., 2011; Koeppe et al., 2012). The enhanced analgesic efficacy of acute THC in Tat(+) mice might be related to alterations in expression of cannabinoid receptors upon Tat induction. Antinociceptive THC effects have been found to be dependent upon CB_1Rs (Craft et al., 2012) and whereas the current study did not assess CB_1R expression levels in the spinal cord, CB_1R levels were significantly upregulated in the prefrontal cortex of Tat(+) female mice. It is known that PLWH use cannabis and/or opioids to cope with pain and stress/anxiety (Potts et al., 2020). Based on the current study and prior literature, findings suggest that PLWH might respond better to cannabis-based interventions compared to opioids for pain treatment even though not all opioids show reduced efficacy in the context of HIV (Palma et al., 2011). Note that in the current study Tat(+) mice did not show altered levels of pain sensitivity to heat-evoked spontaneous nociception with vehicle exposure. Neuropathic pain is commonly reported in PLWH (Lu et al., 2021; Slawek, 2021) and mouse models have demonstrated allodynia and damage to nerve fibers resulting from induced Tat expression (Chi et al., 2011; Wodarski et al., 2018; Cirino et al., 2021). However, Tat effects on nociception are complex and highly dose- and time-dependent (Bagdas et al., 2020). Similar to the current study, in the absence of drug treatment, a previous study reported no effects on heat-evoked nociception when Tat was induced for less than a month (Fitting et al., 2012). Other studies have suggested that the excitatory and neurotoxic effects of Tat on

nociceptive neurons (Chi et al., 2011; Wodarski et al., 2018) may produce first a period of induced dysfunction (i.e., diminishment of pain signaling) followed by pain hypersensitivity as neuronal damage increases (Bagdas et al., 2020). Overall, the findings on nociception provide support for the capacity of acute THC administration to reduce pain and potentially with higher efficacy in the context of neuroHIV.

Besides the use of cannabis for pain management, the prevalence of comorbid depressive and anxiety disorders in PLWH is high (Whetten et al., 2008; Mannes et al., 2021; Liu et al., 2022; Mitra and Sharman, 2022). Literature has reported that cannabis use can mitigate the displayed anxiety in PLWH and decrease stress-related events (Prentiss et al., 2004; Woolridge et al., 2005). Nevertheless, cannabis use has also been reported to increase anxiety as a secondary effect (Costiniuk et al., 2019) and can further lead to the development of panic disorders (Dannon et al., 2004), especially in women (Sholler et al., 2021). THC has been identified as the main component contributing to the cannabis-induced anxiogenic effects (Kasten et al., 2019; Madras, 2019; Raymundi et al., 2020). In the current study Tat induction did not affect anxiety, even though increased anxiety-like behavior has been reported for the Tat transgenic mouse model, potentially due to longer durations of Tat induction (i.e. up to 3 months) and at higher doses (i.e. via DOX injections instead of DOX food) (Paris et al., 2014; Kasten et al., 2017; Joshi et al., 2020; Salahuddin et al., 2020; Qrareya et al., 2021). Further, females compared to male mice demonstrated higher anxiety-like behavior independent of Tat or THC exposure, which is supported in various human studies (Bahrami and Yousefi, 2011; McLean et al., 2011). Interestingly, acute THC increased anxiety in control Tat(−) females and males but not in Tat(+) mice. Previous work in animal models have shown that tests of anxiety-like behavior, including the elevated plus maze task, show highly dose- and route-dependent effects of THC, with lower or inhaled doses associated with anxiolytic action (Bruijnzeel et al., 2016; Black et al., 2019) and higher or injected doses (i.e. 10 mg/kg THC) associated with anxiogenic effects (Dannon et al., 2004; Kasten et al., 2019; Schep et al., 2020). Interestingly, in the presence of Tat the displayed anxiogenic effects of acute THC disappeared, which stays in contrast to the finding of higher efficacy of acute THC on nociception with Tat induction. The decrease of dendritic spine density in the amygdala upon Tat induction reported by a recent study (Nass et al., 2022) might contribute to the disruption of THC's effect on anxiety-like behavior, and thus maybe CNS-dependent. Additionally, whereas acute THC did not downregulate locomotor activity at any of the given doses, exploration behavior assessed in the novel object recognition task demonstrated decreased exploration of the presented objects for Tat(−) mice in the presence of THC, but not for Tat(+) mice. Thus, potential decreased exploratory movements around the elevated plus maze might have resulted in less pokes into the open arms and higher apparent anxiety for Tat(−) compared to Tat(+) mice. Overall, the present data indicate that, while one month Tat induction may not increase anxiety, PLWH may experience less of the anxiogenic effects exerted by higher doses of acute THC compared to the general population.

It is known that around 30–50% of PLWH display HAND complications related to the prefrontal cortex, including memory and learning deficits (Cysique et al., 2004; Garvey et al., 2009; Heaton et al., 2011). Research has shown that prefrontal cortex-related tests, including the novel object recognition task, typically show recognition memory deficits in mice exposed to Tat (Carey et al., 2012; Marks et al., 2016)

or exposed to high doses of CB_1R agonists, including THC (Clarke et al., 2008; Sticht et al., 2015; Barbieri et al., 2016; Zhang et al., 2016; Kasten et al., 2017; Mouro et al., 2018; Ito et al., 2019). The present study supports both findings, which are however specific to female mice. No effects were noted for males, which might be due to the similarity of chosen objects (Frick and Gresack, 2003; Cyrenne and Brown, 2011) or the lack of changes seen in the endocannabinoid system. Specifically in female mice, acute THC exposure appeared to have differential effects on object exploration preference. Whereas, in Tat(−) females the natural tendency of exploring the novel object was reversed with seeking out the familiar object, THC did not affect recognition memory in Tat(+) females. The differential effects of acute THC exposure in female mice might be due to an altered endocannabinoid system in the prefrontal cortex upon Tat induction which was not noted in males.

Upregulated expression levels for AA, CB_1R, FAAH, and a non-significant trend for AEA levels, were demonstrated in the prefrontal cortex of Tat(+) females compared to Tat(−) females. Changes of the endocannabinoid system in the brain of PLWH have been reported (Cosenza-Nashat et al., 2011; Swinton et al., 2021). Specifically, CB_1R and CB_2R expression levels were shown to be altered in the frontal lobe of PLWH with HAND or encephalitis (Cosenza-Nashat et al., 2011; Swinton et al., 2021). Upregulated CB_1R levels in the frontal cortex of PLWH with HAND compared to neurocognitively unimpaired PLWH was shown to be associated with worse cognitive performance (Swinton et al., 2021). Similarly, a previous study found upregulated CB_1R levels in the infralimbic region of the prefrontal cortex in Tat(+) females to be associated with behavioral deficits in an inhibitory control task (Jacobs et al., 2019). Whereas the current study did not find an association with upregulated CB_1R levels in the Tat(+) female mice and cognitive performance, object recognition deficits were significantly associated with increased AEA levels in the prefrontal cortex of Tat(+) females. This was accompanied by a significant increase in the FAAH enzyme, that is responsible for the degradation of AEA to AA and ethanolamine, with AA also being upregulated in the Tat(+) females prefrontal cortex. AA is known to be an important player in pro- and anti-inflammatory responses (Bosetti, 2007; Tallima and El Ridi, 2018; Wang et al., 2021). Upregulated AEA levels in the prefrontal cortex of Tat(+) females compared to Tat(−) females have been demonstrated previously (Xu et al., 2022). Even though the breakdown of AEA contributes to the production of inflammatory lipid mediators, such as AA, it is suggested that the observed upregulation of the endocannabinoid system in Tat(+) female mice is a compensatory response to the Tat-induced upregulation of inflammatory tone and observed behavioral deficits. Attenuation of neuroinflammation by increasing endocannabinoid levels has been demonstrated via the direct application of 2-AG or AEA or by inhibiting the breakdown of 2-AG and AEA via enzyme inhibitors such as MAGL or FAAH (Krishnan and Chatterjee, 2014; Avraham et al., 2015; Zhang and Thayer, 2018).

Besides the effects of Tat induction on the endocannabinoid system, sex was demonstrated to affect the endocannabinoid system and related lipids, with females showing increased 2-AG, AEA, AA, CB_2R, MAGL, and FAAH levels compared to male mice in various brain regions (except for the spinal cord, opposite for AA). Not much is known about sex-dependent effects on the endocannabinoid system in PLWH and its relation to neuroinflammation and HAND. In general, women appear to be more vulnerable to HAND

symptoms (Maki and Martin-Thormeyer, 2009; Maki et al., 2018; Sundermann et al., 2018; Rubin et al., 2019; Duarte et al., 2021) and demonstrate higher levels of immune activation (Ziegler and Altfeld, 2016; Santinelli et al., 2020). For cannabis use, women are typically found to be more sensitive to cannabis (Cooper and Craft, 2018; Matheson et al., 2020; Sholler et al., 2021) which is supported by animal studies for THC (Blanton et al., 2021). It is known that chronic HIV infection in the brain elicits neuroinflammatory responses which significantly contributes to synaptodendritic damage and neuronal dysfunction and thus, HAND (Gartner, 2000; Kaul et al., 2001; Harezlak et al., 2011). Similar to our findings of increased AA levels in the prefrontal cortex of Tat(+) females compared to Tat(−) females, a recent study reported the upregulation of the AA cascade and eicosanoid production in the brains of gp120 transgenic mice with sexual dimorphic transcription of genes related to the two AA pathways, cyclooxygenase and lipoxygenase (Yuan et al., 2022). Interestingly, whereas acute THC did not alter AA levels or the endocannabinoid system in the present study, it is known that chronic cannabis use can lower neuroinflammation in PLWH and neuroHIV animal models (Ellis et al., 2020; Watson et al., 2021). The anti-inflammatory effects of cannabinoids in the context of neuroHIV have been shown to be associated with a CB_2R activation (Cabral and Griffin-Thomas, 2009; Persidsky et al., 2011; Costantino et al., 2012; Hu et al., 2013; Avraham et al., 2014; Purohit et al., 2014). Multiple studies demonstrate increased expression levels of CB_2Rs in HIV (Benito et al., 2005; Cosenza-Nashat et al., 2011; Ramirez et al., 2013), specifically due to activation of microglia (Ashton and Glass, 2007; Benito et al., 2008; Basu and Dittel, 2011; Miller and Devi, 2011; Concannon et al., 2016). Even though CB_2R expression was not altered by Tat induction in the current study, Tat(+) females demonstrated upregulated CB_1R levels in the prefrontal cortex. Upregulated CB_1R levels in the frontal lobe of PLWH with HAND was demonstrated to be localized on glia (Swinton et al., 2021). This is interesting and may point to THC's effects in Tat(+) females being specifically related to its anti-inflammatory effects on glia via upregulated CB_1R. Lastly, the higher sensitivity of females to THC might be related to sex differences in metabolizing THC levels. The current study showed higher THC-COOH concentrations in the plasma of females compared to male mice, which is supported in the literature (Claus et al., 2020) and has shown to have anti-inflammatory and antinociceptive properties (Doyle et al., 1990).

5. Conclusion

As cannabis use is highly prevalent in PLWH, the present study aimed to characterize the acute effects of THC in a neuroHIV mouse model on behavior and the endocannabinoid system. Findings indicate that acute THC has differential effects on various behavioral outcomes in the context of HIV which appear to be sex dependent. Besides the need to investigate the effects of chronic THC in neuroHIV to determine the limiting and beneficial effects of chronic cannabis use in PLWH, future studies should also assess THC in combination with CBD to more accurately approximate real-life use and to directly understand the implications of various CBD:THC ratios.

Abbreviations

2-AG
2-arachidonoylglycerol
AA
arachidonic acid
AEA
anandamide/N-arachidonoylethanolamine
ANOVA
analysis of variance
ART
antiretroviral therapy
CBD
cannabidiol
CB_1R
cannabinoid receptor type 1
CB_2R
cannabinoid receptor type 2
CNS
central nervous system
DOX
doxycycline
EPM
elevated plus maze
FAAH
fatty acid amide hydrolase
GFAP-rtTA
reverse tetracycline transactivator driven by the glial fibrillary acidic protein promoter
HAND
HIV-associated neurocognitive disorders
HIV-1
human immunodeficiency virus type 1
MAGL
monoacylglycerol lipase
NOR
novel object recognition
OEA
oleoylethanolamide
PEA
palmitoylethanolamide
PLWH
people living with HIV

s.c.
subcutaneous, with reference to an injection
SEM
standard error of the mean
Tat
transactivator of transcription
THC
Δ^9-tetrahydrocannabinol
°C
degrees Celsius

Declarations

Author Contributions

Conceptualization, B.J.Y-S., W.J., and S.F.; Methodology, B.J.Y-S., W.J., and S.F.; Validation, B.J.Y-S., B.L.G., W.J., and S.F.; Formal Analysis, B.J.Y-S., B.L.G., W.J., and S.F.; Investigation, B.J.Y-S., B.L.G., H.D., S.R., and S.F.; Resources, W.J., and S.F.; Data Curation, B.J.Y-S., B.L.G., H.D., S.R., D.W., W.J., and S.F.; Writing – Original Draft Preparation, B.J.Y-S., B.L.G., and S.F.; Writing – Review & Editing, B.J.Y-S., B.L.G., H.D., S.R., W.J., and S.F.; Visualization, B.J.Y-S., B.L.G., and S.F.; Supervision, B.J.Y-S., W.J., and S.F.; Project Administration, B.J.Y-S., B.L.G., W.J., and S.F.; Funding Acquisition, W.J., and S.F.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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Consent to participate

Not applicable.

Consent to publication

Not applicable.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Data availability

The data that support the findings of this study are available from the corresponding author (Sylvia Fitting) upon request.

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Figures

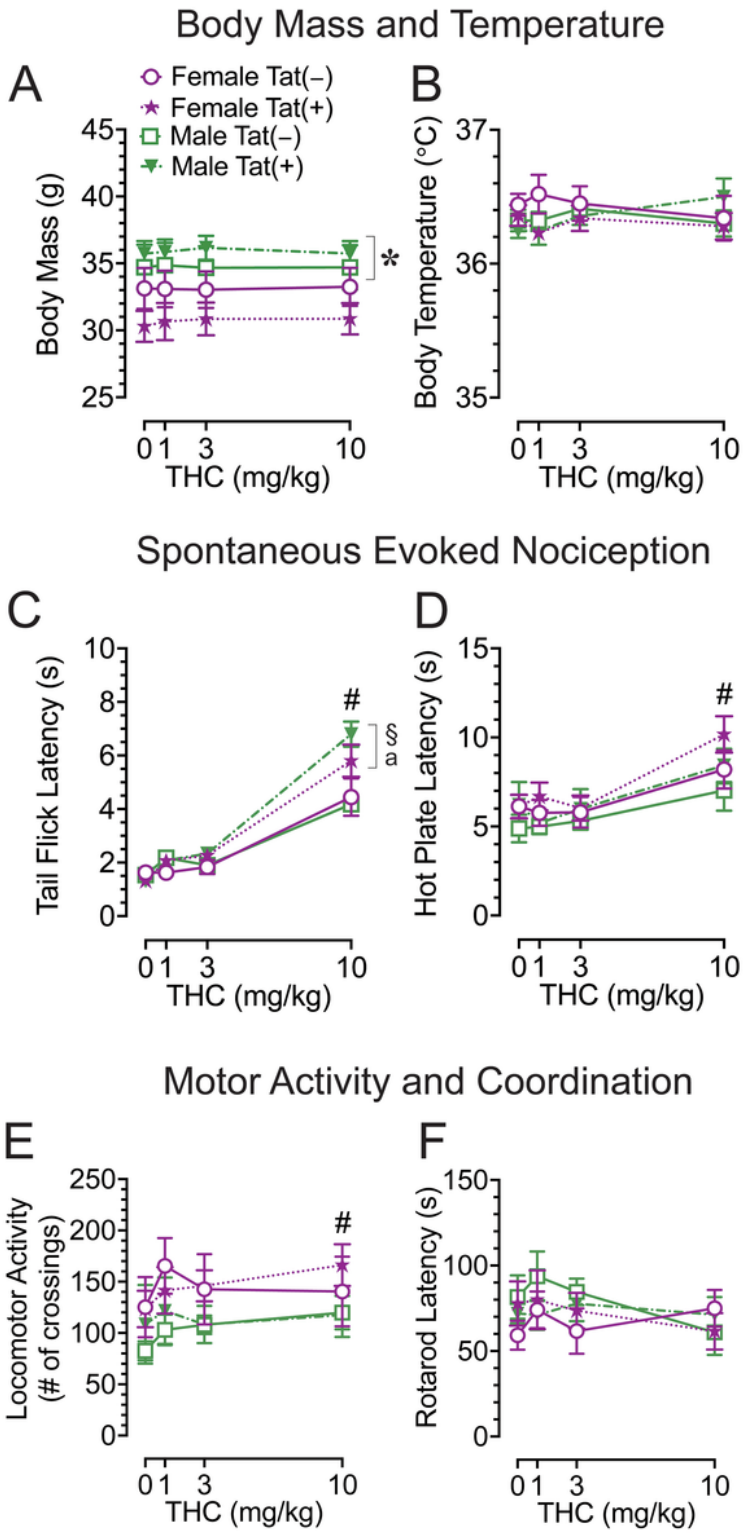

Figure 1

Effects of acute THC doses (0, 1, 3, and 10 mg/kg) on body mass, body temperature, spontaneous evoked nociception, and motor function. (A & B) Body mass and body temperature are not affected by acute THC doses. **(A)** Body mass was significantly higher for male mice compared to females with no significant difference between Tat(−) and Tat(+) animals. No effects were noted for administration of acute THC doses (1 – 10 mg/kg). **(B)** No effects were noted for body temperature. **(C & D)** Spontaneous

heat-evoked nociception is significantly decreased by THC doses and in the tail-flick assay altered by Tat induction. **(C)** For the spinal-related tail-flick assay THC administration at 3 and 10 mg/kg increased latency to pain signals. Importantly, 3 and 10 mg/kg doses of THC showed enhanced antinociceptive effects in Tat(+) mice compared to Tat(−) animals. **(D)** For the supraspinal-related hot-plate assay THC administration at 10 mg/kg increased latency to pain signals but genotype or sex did not alter latency response. **(E & F)** Locomotor activity is increased by acute THC administration without affecting motor coordination. **(E)** Administration of all THC doses increased locomotor activity compared to vehicle exposure. **(F)** No effects or interactions were noted for motor coordination assessed with the rotarod task. All data are expressed as mean ± the standard error of the mean (SEM). Statistical significance was assessed by ANOVAs followed by Bonferroni's post hoc tests when appropriate; $^{*}p = 0.014$ main effect of sex; $^{\#}p < 0.05$ main effect of drug dose; $^{\S}p < 0.001$ main effect of genotype; $^{a}p < 0.001$ drug dose x genotype interaction. n = 9-10 mice per group.

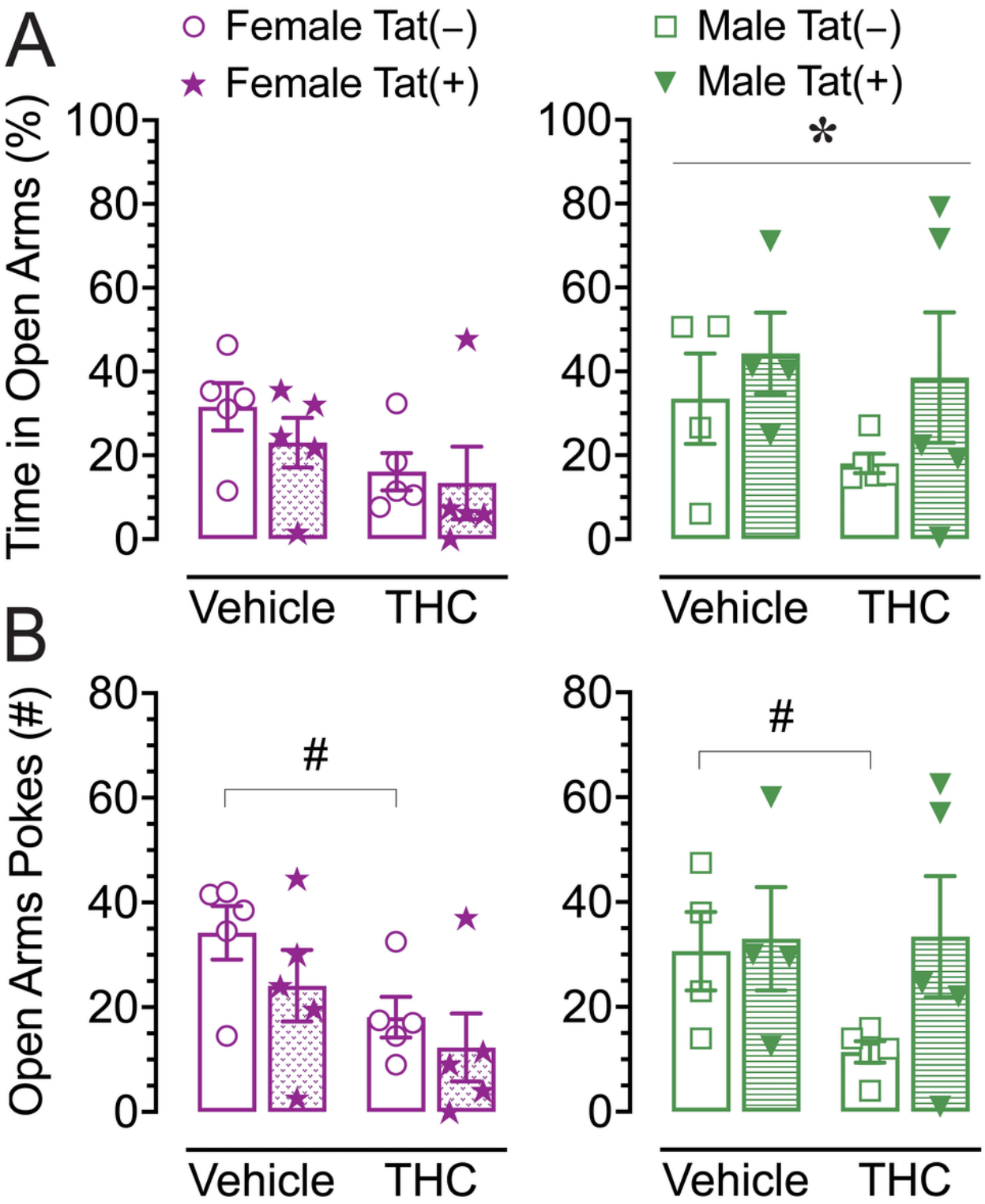

Figure 2

Anxiety-like behavior assessed by the elevated plus maze was altered by sex and acute THC (10 mg/kg) exposure. **(A)** Female mice spent less time in the open arms compared to males, demonstrating more anxiety-like behavior. **(B)** THC administration increased anxiety-like behavior, specifically in Tat(−) female and Tat(−) male mice. All data are expressed as mean ± the standard error of the mean (SEM). Statistical

significance was assessed by ANOVAs; *p = 0.050 main effect of sex; $^{\#}p$ < 0.05 main effect of drug dose. THC dose = 10 mg/kg. n = 4-5 mice per group.

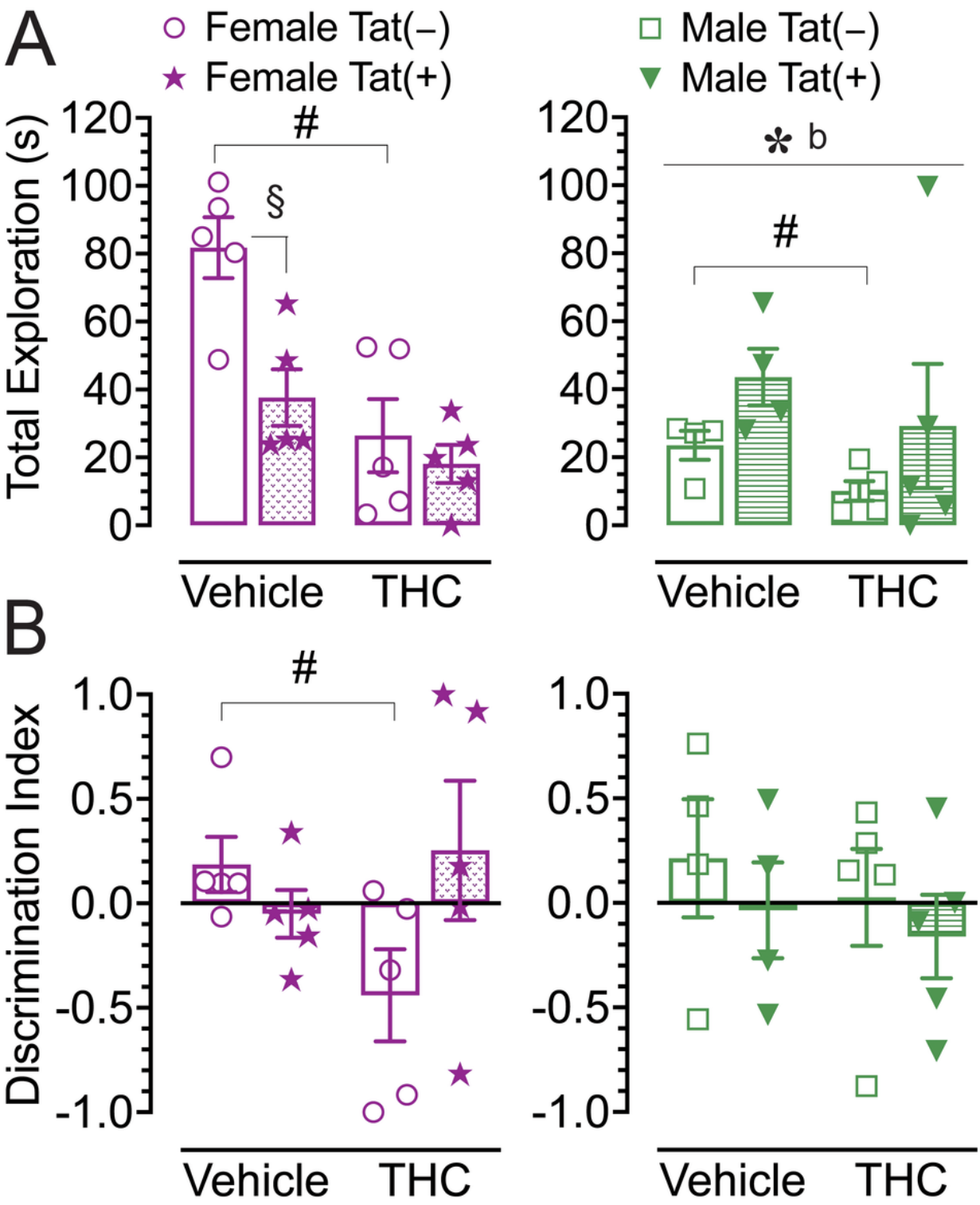

Figure 3

Novel object recognition memory was differentially altered by acute THC (10 mg/kg) exposure in Tat(−) females compared to Tat(+) female mice without affecting males. **(A)** Total object (novel and familiar

objects) exploration time was significantly altered by THC administration, genotype, and sex. **(B)** Novel object recognition memory, indicated by the discrimination index (0 = no preference, 1 = complete preference for the novel object, -1 = complete preference for the familiar object), was differentially altered by THC administration in female Tat(−) compared to female Tat(+) mice. No effects were noted for males. All data are expressed as mean ± the standard error of the mean (SEM). Statistical significance was assessed by ANOVAs; *p = 0.049 main effect of sex; $^{\#}p < 0.05$ main effect of drug dose; $^{\S}p$ = 0.007 main effect of genotype; ^{b}p = 0.003 sex x genotype interaction. THC dose = 10 mg/kg. n = 4-5 mice per group.

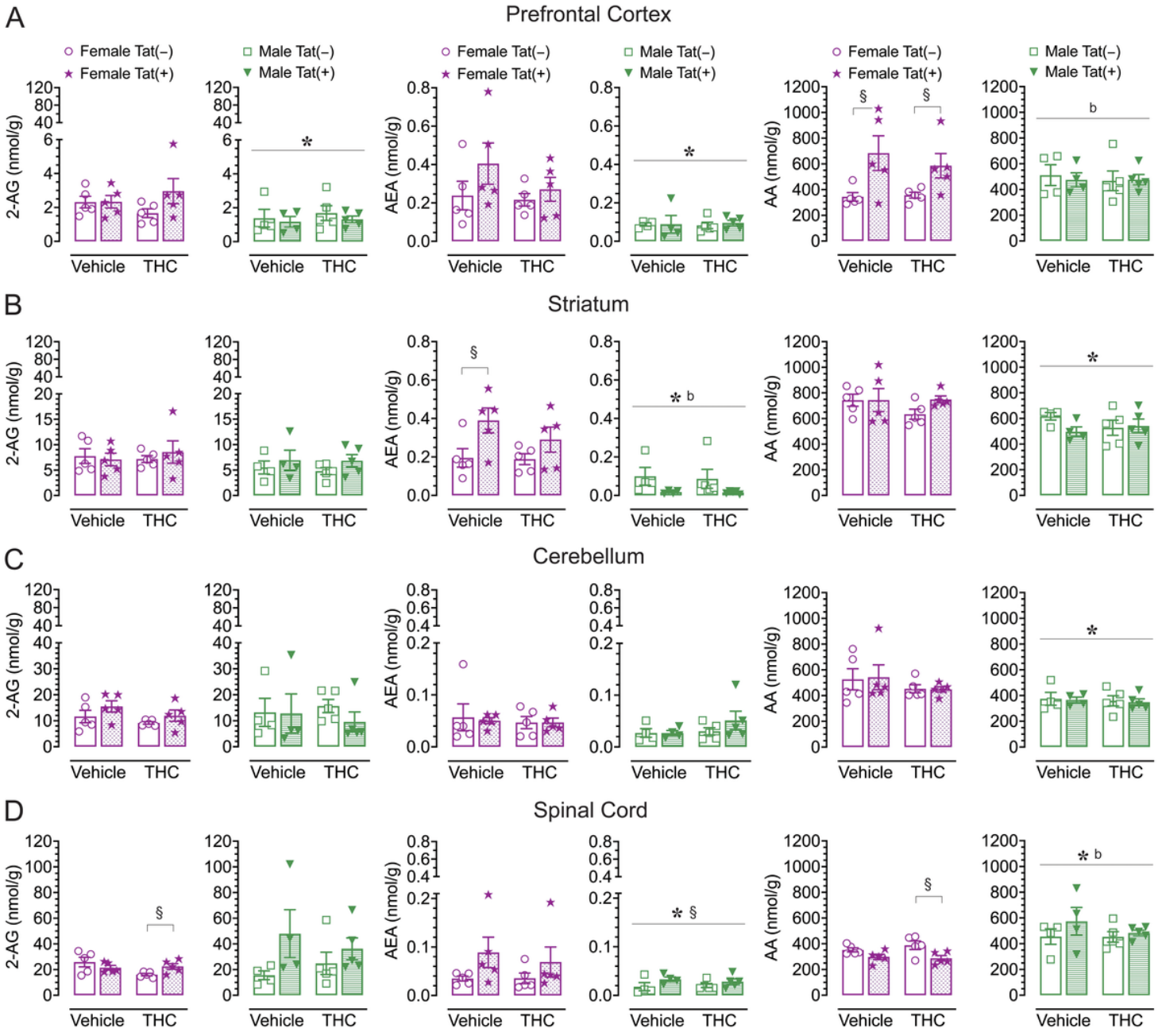

Figure 4

AEA and AA levels (nmol/g) were not affected by acute THC (10 mg/kg) exposure in various CNS regions but showed differences for sex and/or genotype. Concentrations of AEA and AA were assessed in the prefrontal cortex, striatum, cerebellum, and spinal cord for vehicle and acute THC-treated Tat transgenic mice via LC/MS/MS. Lipid concentrations were normalized to nmol/g of tissue. Note that no effects or interactions were noted for acute THC administration on any measure. **(A)** In the prefrontal cortex, female mice showed higher 2-AG and AEA levels compared to male mice and AA levels were altered by genotype and sex with Tat(+) females showing higher AA levels compared to Tat(−) females, but no difference was noted for males. **(B)** In the striatum, no significant effect or interaction was noted for 2-AG but female mice showed higher AEA and AA levels compared to male mice. For AEA, a sex x genotype interaction indicated that Tat(+) females showed higher AEA levels compared to Tat(−) females, which was specific to the vehicle-exposed females. **(C)** In the cerebellum, no effects were noted for 2-AG or AEA, but female mice showed higher AA levels compared to male mice. **(D)** In the spinal cord, a genotype effect was noted for 2-AG, which was specific to THC-exposed female mice, with Tat(+) females showing higher 2-AG levels compared to Tat(−) females. For AEA, female mice showed higher AEA levels compared to male mice, and AEA expression was upregulated for Tat(+) mice compared to Tat(−) mice. For AA, a sex effect demonstrated higher AA levels for males compared to females. Additionally, a sex x genotype interaction indicated that Tat(+) females showed lower AA levels compared to Tat(−) females, which was specific to the THC-exposed females. All data are expressed as mean ± the standard error of the mean (SEM). Statistical significance was assessed by ANOVAs; *$p < 0.05$ main effect of sex; $^{\S}p < 0.05$ main effect of genotype; $^{b}p < 0.05$ sex x genotype interaction. THC dose = 10 mg/kg. n = 4-5 mice per group.

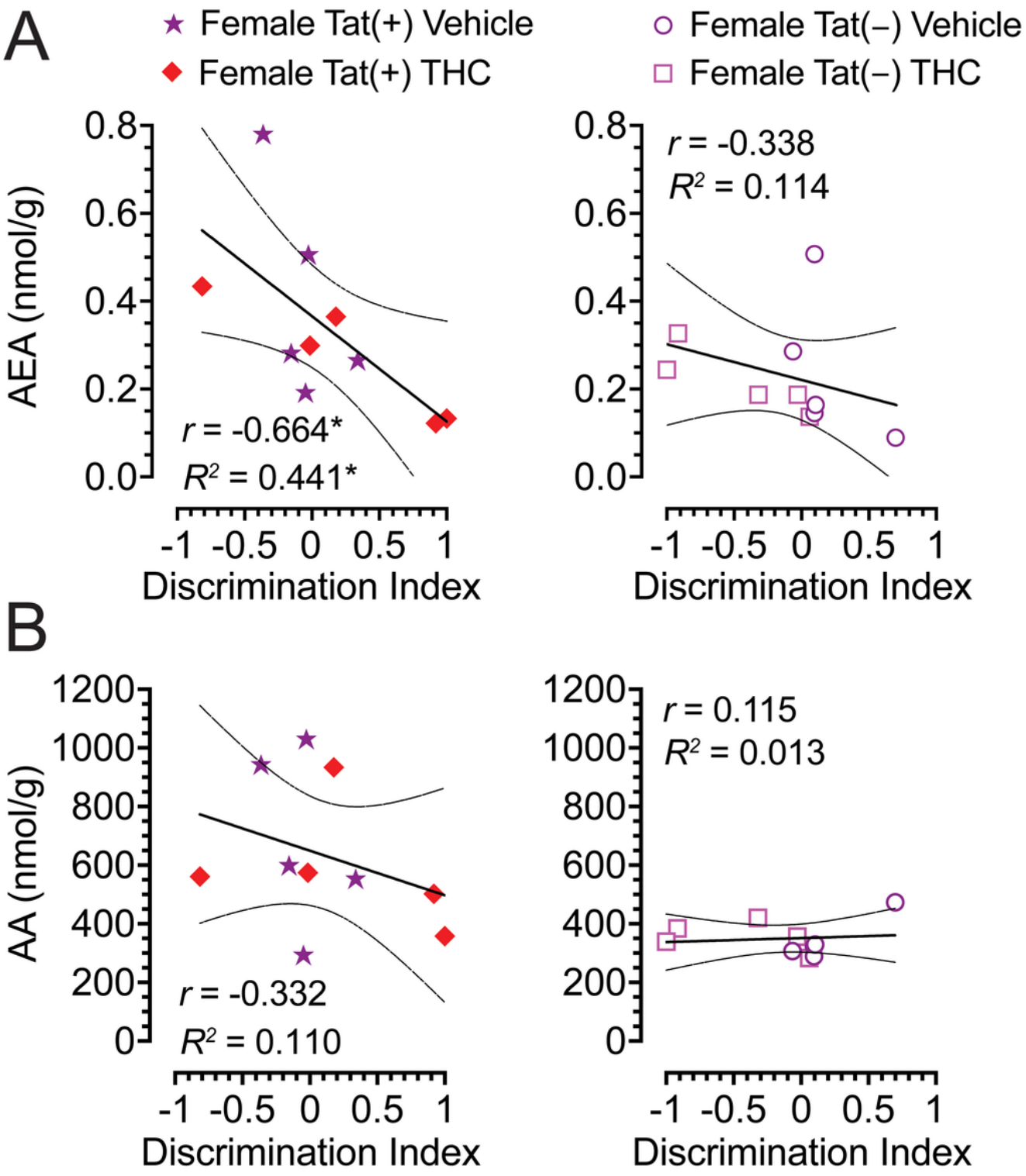

Figure 5

AEA levels in the prefrontal cortex predicts object recognition memory of Tat(+) female mice. Pearson correlation analyses were followed by simple regression analyses in female Tat(+) and female Tat(−) mice to explore the relationship between AEA and AA in the prefrontal cortex and object recognition memory, assess by the discrimination index. **(A)** A significant negative correlation was noted for Tat(+) females with low AEA levels being associated with better object recognition memory. A simple linear

regression demonstrated that AEA levels in the prefrontal cortex accounted for 44% of total variance in the object recognition memory data. No significant effects were noted for Tat(−) females. (**B**) No association was found between AA levels in the prefrontal cortex and object recognition memory in Tat(+) and female Tat(−) mice. *$p < 0.05$

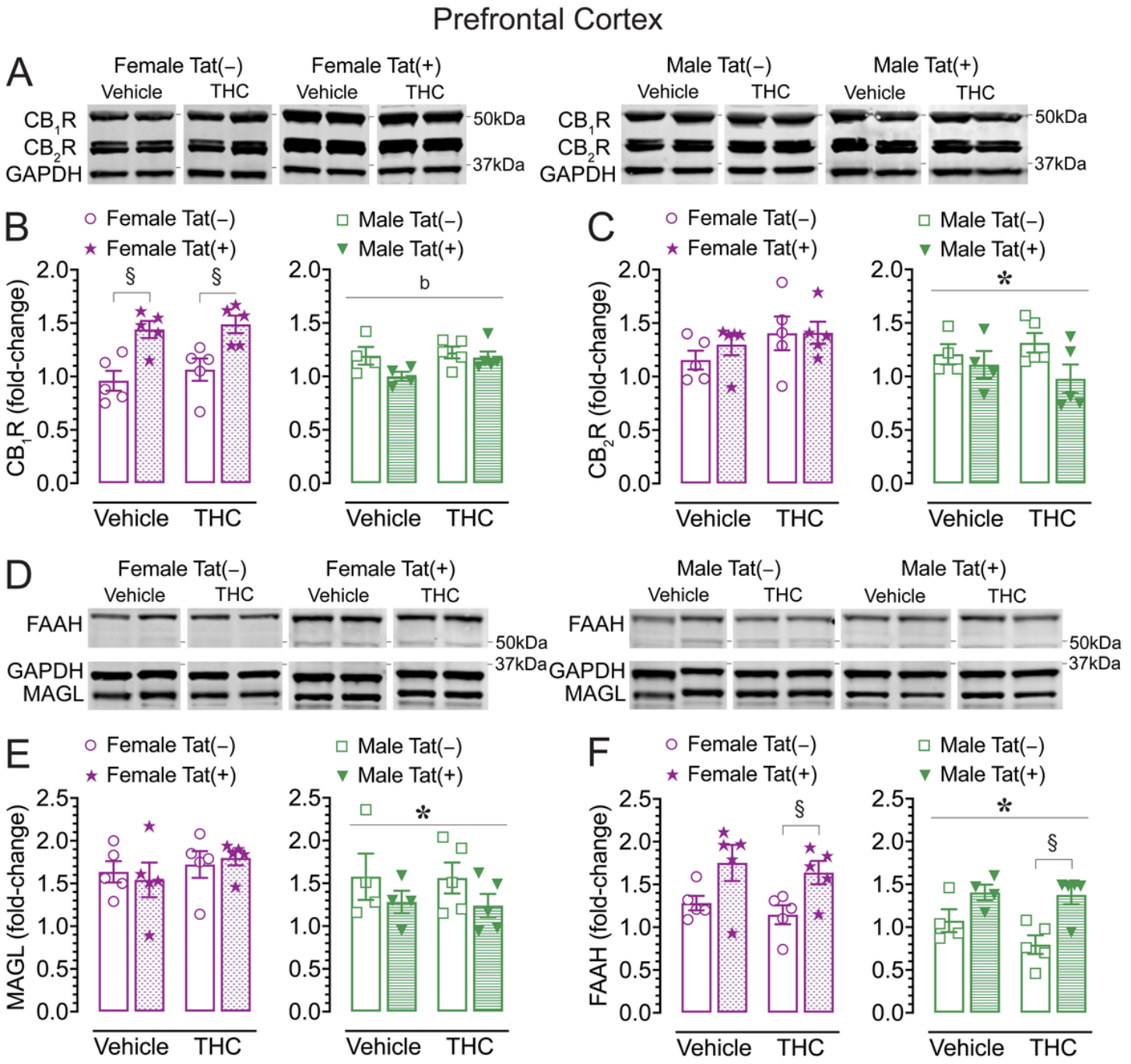

Figure 6

CB_1R, CB_2R, MAGL and FAAH expression levels in the prefrontal cortex were significantly altered by sex and/or genotype. CB_1R, CB_2R, MAGL and FAAH expression levels were assessed in the prefrontal cortex of vehicle and acute THC-treated Tat transgenic mice via Western blot. Data were normalized to the

housekeeping protein GAPDH and fold-change was calculated using a control sample represented on all blots. **(A)** Representative immunoblots for CB_1R, CB_2R, and GAPDH. **(B)** CB_1R protein expression was significantly upregulated in Tat(+) females compared to Tat(−) females, without affecting male mice. **(C)** CB_2R expression was increased in females compared to male mice. **(D)** Representative immunoblots for FAAH, MAGL, and GAPDH. **(E)** Similarly, MAGL expression was upregulated in female mice compared to males. **(F)** FAAH expression was upregulated in females compared to males with THC-exposed Tat(+) females and THC-exposed Tat(+) males demonstrating higher FAAH levels compared to their THC-exposed Tat(−) counterparts. All data are expressed as mean ± the standard error of the mean (SEM). Statistical significance was assessed by ANOVAs; *$p < 0.05$ main effect of sex; $^{\S}p < 0.05$ main effect of genotype; $^{b}p < 0.05$ sex x genotype interaction. THC dose = 10 mg/kg. n = 4-5 mice per group.

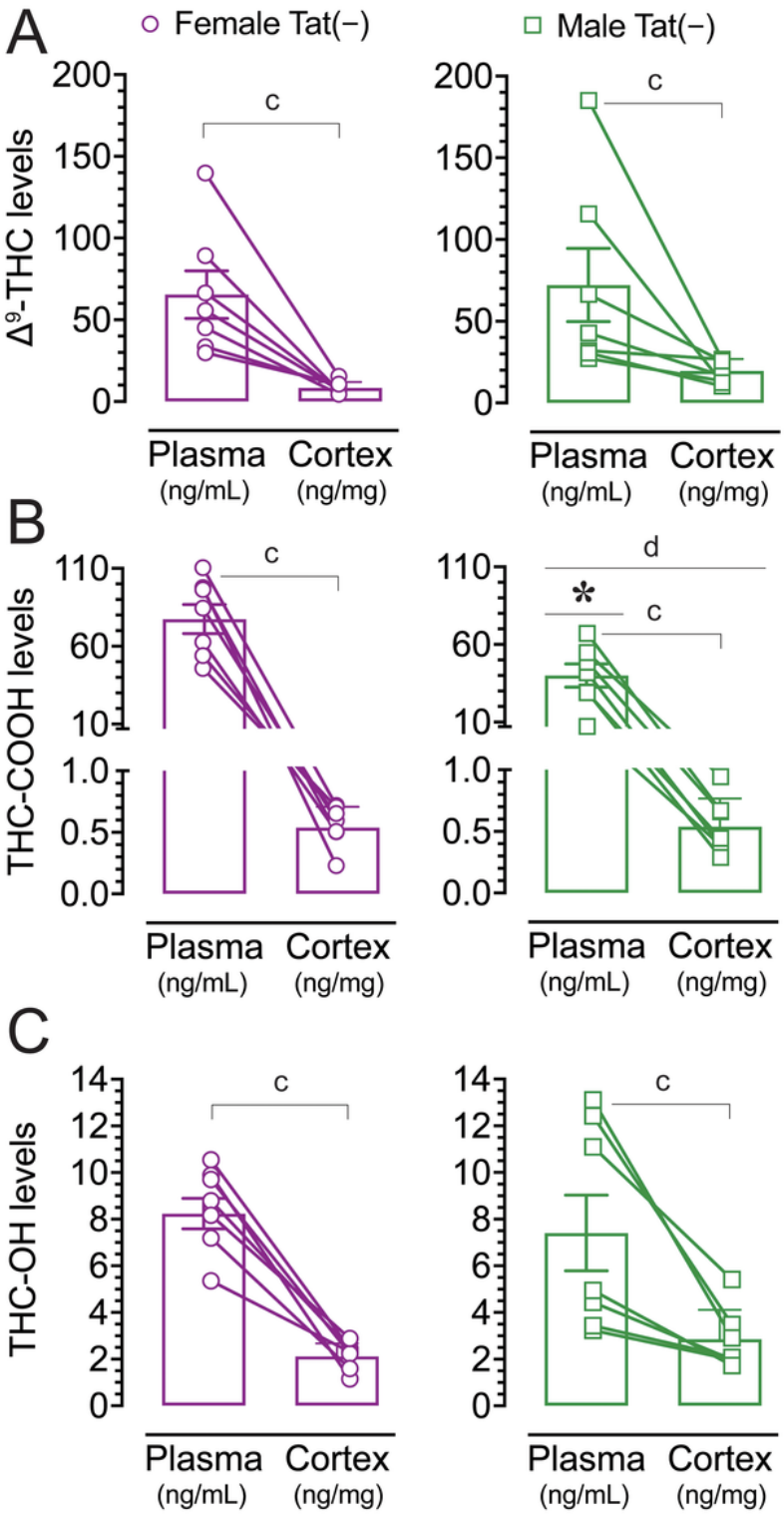

Figure 7

Δ -THC levels and its metabolites after acute THC (10 mg/kg) exposure in plasma and cortex. Concentrations of Δ -THC levels and its metabolites were assessed 1 h after 10 mg/kg THC administration. Concentrations are expressed as ng/mL for plasma and ng/mg for cortex samples. Concentrations for all measures were higher in the plasma compared to the cortex. **(A)** Δ -THC levels were higher in the plasma compared to the cortex in females and males. No other effect was significant.

(B) THC-COOH levels were higher were higher in the plasma compared to the cortex in females and males. Further, a significant sex effect and sample x sex interaction were noted, with females displaying higher THC-COOH levels in the plasma compared to males, but no sex effect was noted for the cortex. **(C)** THC-OH levels were higher in the plasma compared to the cortex in females and males. No other effect was significant. All data are expressed as mean ± the standard error of the mean (SEM). Statistical significance was assessed by ANOVAs; *p = 0.009 main effect of sex; [c]p £ 0.05 main effect of sample; [d]p = 0.008 main effect of sample; THC dose = 10 mg/kg. n = 7 mice per group.

Supplementary Files

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