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Δ^9-Tetrahydrocannabinol alleviates hyperalgesia in a humanized mouse model of sickle cell disease

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Abstract: People with sickle cell disease (SCD) often experience chronic pain as well as unpredictable episodes of acute pain, which significantly affect their quality of life and life

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expectancy. Current treatment strategies for SCD-associated pain primarily rely on opioid analgesics, which have limited efficacy and cause serious adverse effects. Cannabis has emerged as a potential alternative, yet its efficacy remains uncertain. In this study, we investigated the antinociceptive effects of Δ^9-tetrahydrocannabinol (THC), cannabis' intoxicating constituent, in male HbSS mice, which express >99% human sickle hemoglobin, and male HbAA mice, which express normal human hemoglobin A, as a control. Acute THC administration (0.1-3 mg-kg^{-1}, intraperitoneal, i.p.) dose-dependently reduced mechanical and cold hypersensitivity in HbSS, but not HbAA mice. In the tail-flick assay, THC (1 and 3 mg-kg^{-1}, i.p.) produced substantial antinociceptive effects in HbSS mice. By contrast, THC (1 mg-kg^{-1}, i.p.) did not alter anxiety-like behavior (elevated plus maze) or long-term memory (24-h novel object recognition). Subchronic THC treatment (1 and 3 mg-kg^{-1}, i.p.) provided sustained relief of mechanical hypersensitivity but led to tolerance in cold hypersensitivity in HbSS mice. Together, the findings identify THC as a possible therapeutic option for the management of chronic pain in SCD. Further research is warranted to elucidate its mechanism of action and possible interaction with other cannabis constituents.

Significance statement: The study explores THC's efficacy in alleviating pain in sickle cell disease (SCD) using a humanized mouse model. Findings indicate that acute THC administration reduces mechanical and cold hypersensitivity in SCD mice without impacting emotional and cognitive dysfunction. Subchronic THC treatment offers sustained relief of mechanical hypersensitivity but leads to cold hypersensitivity tolerance. These results offer insights into THC's potential as an alternative pain management option in SCD, highlighting both its benefits and limitations.

Introduction

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Sickle cell disease (SCD) is a hereditary disorder caused by a mutation in the hemoglobin gene, resulting in the distortion of red blood cells into a characteristic crescent shape (Ware et al., 2017). Deformed cells adhere to the vascular endothelium and other blood cells, leading to microvascular occlusions and unpredictable, often recurrent episodes of acute pain (vasoocclusive crises). The severity and unpredictability of such episodes make it the primary driver of poor quality of life, frequent hospitalizations, and shortened life expectancy in people with SCD (Ballas & Lusardi, 2005; Platt et al., 1994; Van Tuijn et al., 2010). Moreover, chronic pain affects a majority of individuals with SCD, often throughout their lives, further exacerbating the disease burden (McClish et al., 2009; Smith et al., 2008). Despite medical interventions that enhance survival, such as hydroxyurea therapy or hematopoietic stem cell transplantation, pain remains a persistent challenge in SCD management (Darbari et al., 2019; de la Fuente et al., 2019; Kohli et al., 2010; Smith et al., 2008; Wong et al., 2014). Opioid analgesics are the standard of care but are only partially effective – possibly owing to SCD-associated changes in their pharmacokinetics (Dampier et al., 1995; Nagar et al., 2004) – and their use is fraught with dose-limiting adverse events, tolerance development, and risk of abuse (Kopecky et al., 2004; Weber et al., 2008, 2013; Yawn et al., 2014). Compounding this, the opioid crisis has ignited clinician hesitancy in prescribing these medications, further compromising SCD pain management in affected individuals (Ruta & Ballas, 2016). Consequently, many people with SCD are seeking alternative treatment options to alleviate pain.

Cannabis is emerging as one such option (Curtis et al., 2020; Frisch, 2014; Howard et al., 2005; Roberts et al., 2018). For example, in a 2018 survey involving 58 adults with SCD, 92% of participants reported that they used cannabis for pain relief, with 79% of them attributing a reduction of their reliance on opioid analgesics to cannabis consumption (Roberts et al., 2018). In addition, retrospective surveys of people with SCD found that cannabis use decreased inpatient hospitalizations (Curtis et al., 2020), and relieved anxiety and depression (Howard et al., 2005). Despite these observations, empirical evidence supporting or contradicting the efficacy of cannabis in SCD pain remains limited and inconclusive (Argueta et al., 2020). Indeed, a pilot randomized crossover clinical trial of vaporized cannabis in 23 individuals with SCD and chronic pain suggested an improvement in mood but did not find a statistically significant reduction of pain and associated symptoms (Abrams et al., 2020).

Cannabis contains numerous biologically active components, with Δ^9-tetrahydrocannabinol (THC) being the most abundant and most extensively studied (Finn et al., 2021; McDonagh et al., 2022). THC produces its pharmacological effects by hijacking the endocannabinoid signaling system (Piomelli & Mabou Tagne, 2022), a critical regulator of nociception and pain, through its

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interaction with CB_1 and CB_2 cannabinoid receptors (Starowicz & Finn, 2017). Nonetheless, THC's activation of CB_1 receptors in brain regions involved in mood, reward, and cognition produces a distinctive set of physiological and psychotropic effects which may limit cannabis application in the management of SCD pain.

In the present study, we investigated the effects of THC in a humanized mouse model of SCD. We evaluated nociceptive responses to mechanical and cold stimuli in male homozygous transgenic mice expressing either human sickle hemoglobin (HbSS mice) or, as a control on the same mixed genetic background, normal human hemoglobin A (HbAA mice). The results indicate that acute THC administration alleviates mechanical and cold hypersensitivity in HbSS mice in a dose-dependent manner without affecting emotional and cognitive dysfunction also present in these mice. Subchronic THC treatment provides sustained relief of mechanical hypersensitivity but leads to tolerance in cold hypersensitivity. The findings shed light on the potential and limitations of THC in managing chronic pain and associated comorbidities in SCD.

Materials and Methods

Study Approval

The study followed ethical guidelines for laboratory animal care set by the National Institutes of Health (NIH) and the International Association for the Study of Pain (IASP). Approval for the study was granted by the Animal Care and Use Committee of the University of California, Irvine.

Animals

We used male homozygous HbSS and HbAA mice aged 5 to 10 months, which were bred and phenotyped as detailed previously (Sagi et al., 2018). HbSS mice, on a mixed genetic background, lack murine α and β globin genes, replaced by human α and β^S globin (Pászty et al., 1997). These mice express > 99% human sickle hemoglobin and feature identifying pathological manifestations of severe SCD, including hemolysis, reticulocytosis, anemia, extensive organ damage, shortened life span, and pain (Hillery et al., 2011; Kohli et al., 2010; Pászty et al., 1997). By contrast, HbAA mice, also on a mixed genetic background without murine α and β globin genes, exclusively express human α and β^A globins, thus producing only normal human hemoglobin A.

Drug preparation and administration

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THC sourced from Cayman Chemicals (Ann Arbor, MI) was freshly prepared prior to administration by evaporating its commercial ethanol solution under a gentle stream of N_2 and dissolving the resulting residue in a vehicle of Tween-80 in sterile saline (5%, vol/vol) (Lee et al., 2024; Torrens et al., 2023). THC and vehicle were administered via intraperitoneal (i.p.) injection at a volume of 10 mL-kg^{-1}.

Behavioral assays

Behavioral Assessment

All experiments were conducted during the light phase of the light/dark cycle. Prior to testing, mice were acclimated to experimental conditions in a quiet and controlled environment. Behavioral assessments were conducted by an experimenter blinded to the treatment protocols.

Mechanical hypersensitivity

Mechanical hypersensitivity was evaluated with a dynamic plantar aesthesiometer (Ugo Basile, Italy) following a 45-min habituation period, as described (Mabou Tagne et al., 2021; Tagne et al., 2021). Withdrawal threshold was defined as the force (in grams) at which mice withdrew their paws from the mechanical stimulus. A 5 g cut-off pressure was set. Three measurements were taken at intervals of 3 min and averaged.

Cold hypersensitivity

Cold hypersensitivity was measured as described (Brenner et al., 2012). Mice were individually placed in small plastic chambers with a glass floor. Following a 2-hour acclimatization period, a compressed dry ice pellet was positioned on the glass surface directly beneath the test hind paw. The latency (in seconds) for the mouse to withdraw its paw was recorded. A 30-s cutoff time was used between each measurement. Two trials were taken and averaged for each paw.

Tail-flick assays

Tail-flick assays were conducted following established protocols (Tagne et al., 2021). Mice were gently restrained in a soft tissue pocket made of the pet training pad (Glad^{TM}), and the distal 1/3 of each mouse's tail was immersed in a hot water bath maintained at 54°C. The latency to withdraw the tail from the bath was recorded (in seconds). Measurements were performed twice, separated by a 5-min interval between trials, and the results were averaged. A 10-s cut-off time was implemented to prevent tissue damage.

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Body temperature

Core body temperature was assessed following established procedures (Lee et al., 2024). Briefly, thermosensitive microchips (2.1 mm × 12 mm, Unified Information Devices, Lake Villa, IL) were implanted into the peritoneum of lightly anesthetized mice. Following a 3-day recovery period in their home cages, mice were given THC or vehicle and immediately transferred to individual cages, where body temperature was recorded using a remote microchip reader at multiple time points over the subsequent 2 hours. Results are presented as the difference (ΔT) between post-injection and baseline temperatures.

Motor coordination

Motor coordination was assessed using an accelerating Rotarod apparatus (Ugo Basile; rod diameter: 3 cm) (Fotio et al., 2024). Mice underwent three consecutive familiarization sessions, during which the speed was gradually increased from 4 to 40 rpm over a 5-minute period. Experiments were conducted 24 hours after the final training session. The time (in seconds) each mouse remained on the rotating rod without falling or looping was recorded.

Catalepsy test

Mice were positioned with both forelegs on a horizontal bar (diameter = 0.5 cm) elevated approximately 4.5 cm above the testing surface. The time (seconds) during which the mice remained immobile on the bar (catalepsy time) was recorded.

Elevated plus maze (EPM) test

The EPM test was performed under mildly aversive environmental conditions, i.e. low ambient light (open arms: 160–180 lux and closed arms: 40–50 lux), following an established protocol (Fotio et al., 2023). Briefly, each mouse was placed on the central platform of the maze, facing closed open arms, and the trial was videotaped for 5 minutes using the Debut video capture software (NCH Software, Canberra, Australia). A blinded observer recorded the time spent in the open and closed arms, as well as the number of entries into each arm type. The anxiety index was calculated using the formula: 1 - [(time spent in open arms/total time) + (open arm entries/total entries)] / 2 (Fotio et al., 2021).

Novel object recognition (NOR) test

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The NOR test was conducted over 3 days (Fotio et al., 2021, 2024). On day 1, the mice were acclimated to the empty arena for 10 minutes. On day 2, they were reintroduced to the arena, which now contained two identical objects, and left there for 10 minutes. On day 3, one of the objects was substituted with a new object of different shape, color, and texture. Mice were given another 10-minute session to explore the arena, during which a blinded observer recorded the total time spent exploring each object (i.e., nosing and sniffing at a distance ≤ 2 cm). The discrimination index was computed as: [(time of novel object exploration) – (time of familiar object exploration)]/total exploration time.

Statistical analyses

Statistical analyses were conducted using GraphPad Prism version 10.2 (La Jolla, CA). Results are presented as means ± SEM of n experiments. Non-linear regression analysis was utilized to calculate ED_{50} values with 95% confidence intervals (CI). The area under the curve (AUC) was determined using the trapezoidal rule, either by computing the net area (subtracting the area of peaks below the baseline from the area of peaks above the baseline) or by summing all the peaks to generate the total area. Statistical significance was set at $P < 0.05$ and assessed using unpaired, two-tailed Student's *t* test or analysis of variance (ANOVA) (one-way or two-way) followed by Dunnett's or Šídák's post hoc tests, as appropriate.

Results

Antinociceptive effects of acute THC administration

Figure 1 illustrates the antinociceptive effects of THC in homozygous HbSS mice, which express human HbS and exhibit distinctive pathological signs of SCD, and HbAA mice, which express human HbA and are phenotypically normal (Sagi et al., 2018; Tran et al., 2021). Confirming previous results (Cherukury et al., 2023; Tran et al., 2021), we found that untreated HbSS mice are hypersensitive to cold (HbSS *vs* HbAA: Δ withdrawal latency = - 9.360 ± 1.385 s; $P < 0.0001$; n = 7-9; Fig. 1A) and mechanical pressure (Δ withdrawal threshold = -1.440 ± 0.413 g; $P = 0.0057$; n = 7-9; Fig. 1C). In a separate group of HbSS mice, we examined the effects of a single i.p. injection of THC (0.1, 0.3, 1 and 3 mg-kg^{-1}, 2 hours before testing). The drug caused a marked, dose-dependent attenuation of cold hypersensitivity (Fig. 1A, B) and mechanical hypersensitivity (Fig. 1C, D), which in both cases was maximal at the dose of 1 mg-kg^{-1}. Median effective dose (ED_{50}) values were 0.45 mg-kg^{-1} (CI 95%: 0.230-0.861) for cold hypersensitivity (Fig. 1B) and 0.43 mg-kg^{-1} (CI 95%: 0.151-1.211) for mechanical

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hypersensitivity (Fig. 1D). No such effect was observed in HbAA mice, in which i.p. administration of THC (1 mg-kg^{-1}, 2 hours before testing) did not alter sensory responses (Fig. 1A, C). A time-course study revealed that the antinociceptive effect of THC on mechanical hypersensitivity was maximal 2 hours after drug injection and lasted for at least 4 hours (Fig. 1F). Finally, we investigated the antinociceptive effects of acute THC administration in the tail-flick assay. HbSS mice were given a single i.p. injection of THC (1 or 3 mg-kg^{-1}) and nociceptive thresholds were measured 1 hour later. Baseline tail withdrawal latencies in vehicle-treated HbSS mice were (mean ± SEM) 5.15 ± 0.81 s (Fig. 1E). Acute THC administration produced significant antinociceptive effects at both doses (Veh *vs* THC 1 mg-kg^{-1}: ∆mean = - 3.07 s, CI 95%: - 5.90 to - 0.25, *P* = 0.03; Veh *vs* THC 3 mg-kg^{-1}: ∆mean = - 3.04 s, CI 95%: - 5.87 to - 0.21, *P* = 0.03; Fig 1E).

Effects of acute THC administration on body temperature and motor activity

To characterize the broader impact of acute THC administration in HbSS and HbAA mice, we measured core body temperature (using thermosensitive microchips) and motor activity (using the Rotarod and bar catalepsy tests), which are affected by activation of CB_1 cannabinoid receptors in the central nervous system (Monory et al., 2007; Zimmer et al., 1999). A single injection of THC (1 mg-kg^{-1}, i.p.) produced a time-dependent hypothermic response in HbSS mice (Veh *vs* THC: Δ AUC = 5.275 ± 1.482 °C × min; *P* < 0.01; Fig. 2A-B) but had no such effect in HbAA control mice (Δ AUC = 0.02500 ± 1.482 °C × min; *P* = 0.9998; Fig. 2A-B). The same THC dose caused no change in Rotarod performance (Fig. 2C-D) and did not cause catalepsy (Table 1) in either HbSS or HbAA mice.

Effects of subchronic THC administration on SCD-induced mechanical and cold hypersensitivity

To determine if the antinociceptive effects of THC persist with repeated administration, we injected the drug at the two highest doses previously tested (1 and 3 mg-kg^{-1}, i.p.) in HbSS mice once daily for 14 consecutive days. Mechanical and cold hypersensitivity were measured 2 and 4 hours after THC or vehicle injection on days 0, 3, 7 and 14 of the experiment (Fig. 3). The results show that administration of 1 and 3 mg-kg^{-1} THC produced a comparable and sustained attenuation of mechanical hypersensitivity, which persisted for at least 14 days (Fig. 3A). By contrast, tolerance to the effects of THC on cold hypersensitivity was evident by day 7, with the 3 mg-kg^{-1} dose, and day 14, with the 1 mg-kg^{-1} dose (Fig. 3B).

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Effects of acute THC administration on anxiety-like behavior and cognition

Pain in SCD is accompanied by various comorbidities, which include anxiety and memory impairment (DeBaun et al., 2020; Karafin et al., 2019). HbSS mice phenocopy these comorbidities and we asked therefore whether THC administration might alleviate them. Separate groups of HbSS and HbAA mice were given acute injections of THC (1 mg-kg^{-1}, i.p.) or vehicle and, 2 hours later, were subjected to either the EPM or the 24-hour NOR test. As anticipated from prior studies (Wang et al., 2016), HbSS mice displayed marked anxiety-like behavior in the EPM test (HbSS *vs* HbAA: Δ anxiety index = 0.3660 ± 0.09046; *P* = 0.0009 *vs* HbAA controls, n = 8-9; Fig. 4A-D) and cognitive deficits in the NOR test (HbSS *vs* HbAA: Δ discrimination index = -0.2247 ± 0.09206; *P* = 0.0442 *vs* HbAA controls, n = 7-8; Fig. 4E). No effect of THC treatment was observed in either test.

Discussion

Surveys suggest that people with SCD self-medicate with cannabis to manage their pain, but empirical evidence supporting or contradicting cannabis' analgesic efficacy in this highly painful condition remains inconclusive. To begin filling this knowledge gap, in the present study we investigated the antinociceptive effects of cannabis' intoxicating constituent, THC, in a humanized mouse model of SCD. The results show that a single injection of THC produces a dose-dependent reversal of mechanical and cold hypersensitivity in HbSS mice, which express sickle human hemoglobin, but not in HbAA mice, which express normal human hemoglobin A on an identical genetic backgound. In HbSS mice, acute THC treatment evoked substantial antinociceptive effects in tail-flick assays. Moreover, subchronic THC treatment provided sustained relief of mechanical hypersensitivity, although tolerance to the relief of cold hypersensitivity developed over time. By contrast, THC did not ameliorate anxiety-like behavior and cognitive deficits associated with both the mouse HbSS phenotype and human SCD. Of note, the antinociceptive effects of THC cannot be attributed to its ability to reduce locomotion (Deuis et al., 2017; Strekalova et al., 2005; Weiss et al., 1998) because rotarod and EPM tests showed that motor coordination (Fig. 2C) and locomotor activity (Fig. 4C) were not affected by THC treatment. The findings identify benefits and limits of THC as a therapeutic option for chronic pain management in people with SCD.

As expected from prior studies in wild-type mice (Nielsen et al., 2022; Piomelli & Mabou Tagne, 2022), THC administration in HbSS mice resulted in significant and dose-dependent antinociceptive effects when administered acutely. Moreover, when given subchronically, THC provided sustained relief of mechanical hypersensitivity, although tolerance developed to the

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effect on cold hypersensitivity over time. The cellular basis for this modality-selective tolerance is unknown. One possible explanation is that CB_1-expressing neurons, which are present in both peripheral and central sensory neurons (Woodhams et al., 2017), might respond differentially to prolonged THC exposure, some developing tolerance and others not. This speculative hypothesis will require empirical evaluation. The results suggest that THC holds promise as a potential analgesic option and may serve as an alternative or a complement to opioids for the management of chronic pain in SCD. The findings align with studies in humanized SCD mice, which showed that the synthetic cannabinoid CP-55940, whose pharmacological actions mimic THC's (Maccarrone et al., 2023), alleviated various aspects of SCD-related pain including chronic and hypoxia-reoxygenation-evoked hyperalgesia, increased sensitivity to touch and temperature extremes, and spontaneous musculoskeletal/deep tissue hyperalgesia (Cain et al., 2012; Kohli et al., 2010; Vincent et al., 2016). Disappointingly, however, a randomized, placebo-controlled crossover trial testing the effects of vaporized cannabis – standardized at a THC/cannabidiol (CBD) ratio of ~1:1 – in patients with SCD reported significant mood improvements but no statistically detectable effect on pain scores (Abrams et al., 2020). Several circumstances may have contributed to this negative outcome, such as patient heterogeneity, small trial size (23 patients), short treatment duration (5 days), and/or selection of an inadequate THC/CBD ratio. Pharmacodynamic factors such as functional interference due to the concomitant use of hydroxyurea and/or opioid analgesics by most participants in the study (Abrams et al., 2020), might have also played a role.

While our study did not directly investigate the mechanism by which THC elicits its antinociceptive effects in HbSS mice, it is plausible that this may involve the activation of CB_1 and/or CB_2 cannabinoid receptors. Most, if not all pharmacological properties of THC are ascribed to its ability to engage these receptors, whose role in pain regulation is well established (Piomelli & Mabou Tagne, 2022; Starowicz & Finn, 2017; Vincent et al., 2016). However, it is important to acknowledge that THC may ligate other receptor systems, such as transient receptor potential vanilloid 2 (TRPV2) (De Petrocellis et al., 2011; Qin et al., 2008), which might also contribute to its antinociceptive activity (Frederick et al., 2007; Shimosato et al., 2005). Further research is needed to fully elucidate the precise molecular mechanisms underlying THC's effects in SCD.

The analgesic response to THC in HbSS mice was accompanied by relatively modest CB_1-mediated side effects, notably a transient hypothermia, but not by changes in motor coordination or motor activity. This is not surprising, because the THC doses used in the present study (0.1-3 mg-kg^{-1}, i.p.) are not typically associated with such effects (Lee et al., 2022;

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Torrens et al., 2020). However, the heightened susceptibility of HbSS mice to THC-induced hypothermia raises concerns about potential risks for individuals with SCD. These risks, including exacerbation of tissue hypoxia, precipitation of vasoocclusive crises, and aggravation of pain symptoms (Ivy et al., 2023), may help explain the observed tolerance to the effects of subchronic THC treatment on cold allodynia. This heightened susceptibility may be attributed to various factors, such as alterations in CB_1 receptor expression or distribution and disease-related changes in pharmacokinetics. Given these potential risks, it is critical to carefully assess the risk-benefit profile of THC when considering its use for pain management in SCD patients.

Pain in SCD is often accompanied by various comorbidities, which include anxiety and memory impairment (Karafin et al., 2019). In our investigation, we aimed to understand if THC, administered at its antinociceptive dose (1 mg-kg^{-1}), could influence these conditions. The results showed, however, that THC administration neither exacerbated nor improved anxiety and memory impairments associated with SCD. These findings should be interpreted in the context of previous evidence suggesting that THC treatment may have both detrimental and beneficial effects on mood and memory function in wild-type mice (Bilkei-Gorzo et al., 2017; Braida et al., 2007; Rubino et al., 2007; Sarne et al., 2018; Schramm-Sapyta et al., 2007). It is plausible that a different dosage or regimen of THC might have led to different outcomes, but further research is needed to test this possibility.

Our study has at least two noteworthy limitations. First, we exclusively investigated humanized SCD mice, which, while having construct, face, and predictive validity, do not fully replicate the complexity of SCD pathology in humans (Kamimura et al., 2024). For instance, unlike patients with SCD, Berkeley and Townes HbSS mice exhibit elevated mean corpuscular volume and low mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration (Kamimura et al., 2024; Pászty et al., 1997; Ryan et al., 1997). Another notable discrepancy is that, unlike human patients with SCD where neutrophils are the predominant white blood cells, lymphocytes predominate in humanized HbSS mice (Kamimura et al., 2024; Szczepanek et al., 2012). Thus, extrapolating findings from this single mouse model to patients with SCD should be approached cautiously. A second limitation of our study is that it focused solely on adult male mice. Since there are sex differences in clinical manifestations among individuals with SCD (Masese et al., 2021), future research involving female mice is needed. Despite these limitations, our results provide support for the use of THC in alleviating pain and improving the quality of life of people living with SCD.

Authorship Contributions.

Participated in research design: Mabou Tagne, Gupta, and Piomelli.

Conducted experiments: Mabou Tagne and Fotio.

Performed data analysis: Mabou Tagne.

Wrote or contributed to the writing of the manuscript: Mabou Tagne, Gupta, and Piomelli.

Data Availability Statement. The authors declare that all the data supporting the findings of this study are contained within the paper.

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Legends for Figures

Figure 1. Antinociceptive effects of THC in HbSS mice. In drug-naïve HbSS mice, the nocifensive responses to cold (A) and mechanical (C) stimuli were increased compared to control HbAA mice. In HbSS mice, THC (0.1, 0.3, 1, and 3 mg-kg^{-1}, i.p.) attenuated cold (A) and mechanical (C) hypersensitivity in a dose-dependent manner but had no such effect in HbAA mice. Vehicle (0 THC) consisted of saline/Tween 80, 95:5 (v/v). (B, D) Log dose-response curves of THC on cold (B) and mechanical (D) hypersensitivity. (E) Withdrawal latencies (in seconds) in tail-flick assays were measured in HbSS mice 60 min after a single i.p. injection of THC (1, 3 mg-kg^{-1}). (F) Time-course of the effects of THC on mechanical hypersensitivity in HbSS mice. Results, presented as mean ± SEM (n = 7-10, each group), were analyzed using one-way ANOVA (A,C,E) or two-way ANOVA (F), followed by Dunnett's or Šídák's post hoc tests, as appropriate. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$ vs control. ns: non-significant.

Figure 2. Effects of THC on body temperature and locomotor coordination. Mice received a single dose of THC (1 mg-kg^{-1}, i.p.) or vehicle (saline/Tween 80, 95:5, v/v), and body temperature (A-B) and latency to fall in the rotarod test (C-D) were measured at various time points. Time-course of the effects of THC on body temperature (A) and rotarod performance (C). The area under the curve (AUC) for body temperature (B) and total peak area for rotarod performance (D) were quantified. Results, presented as mean ± SEM (n = 6, each group), were analyzed using one- (B and D) or two-way ANOVA (A and B), followed by Šídák's or Tukey's post hoc tests, as appropriate. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$ vs control. ns: non-significant.

Figure 3. Antinociceptive effects of subchronic THC administration in HbSS mice. Mice received once daily injections of THC (1, 3 mg-kg^{-1}, i.p.) or vehicle (saline/Tween 80, 95:5, v/v) for 2 weeks. Mechanical (A) and cold (B) hypersensitivity were measured 4h or 2h after THC injections, respectively. Results, presented as mean ± SEM (n = 5-8, each group), were analyzed using two-way ANOVA followed by Dunnett's post hoc tests, as appropriate. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$ vs control. ns: non-significant.

Figure 4. Effects of a single THC injection (1 mg-kg^{-1}, i.p.) on anxiety-like behavior (A-D) and memory deficits (E) in HbSS mice. Compared to HbAA control, HbSS mice spend more time in the closed arms (A) and less time in the open arms (B) of the EPM. Their total arm entries (C) were unaffected. Their anxiety index (D) is markedly higher. In addition, SCD is associated with worse cognitive performance in the NOR test (E). A single dose of THC does not alter these

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behaviors 2h post-injection. Results, presented as mean ± SEM (n = 7-9, each group), were analyzed using one-way ANOVA, followed by Dunnett's post hoc tests. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$ vs control. ns: non-significant.

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Legends for Tables

Table 1. Catalepsy time after THC treatment (1 mg-kg^{-1}, i.p.) in HbSS and HbAA mice (n = 6 per group).

	HbSS - Veh	HbAA - THC	HbAA - Veh	HbSS - THC
Catalepsy time (s)	0	0	0	0

Figure 1

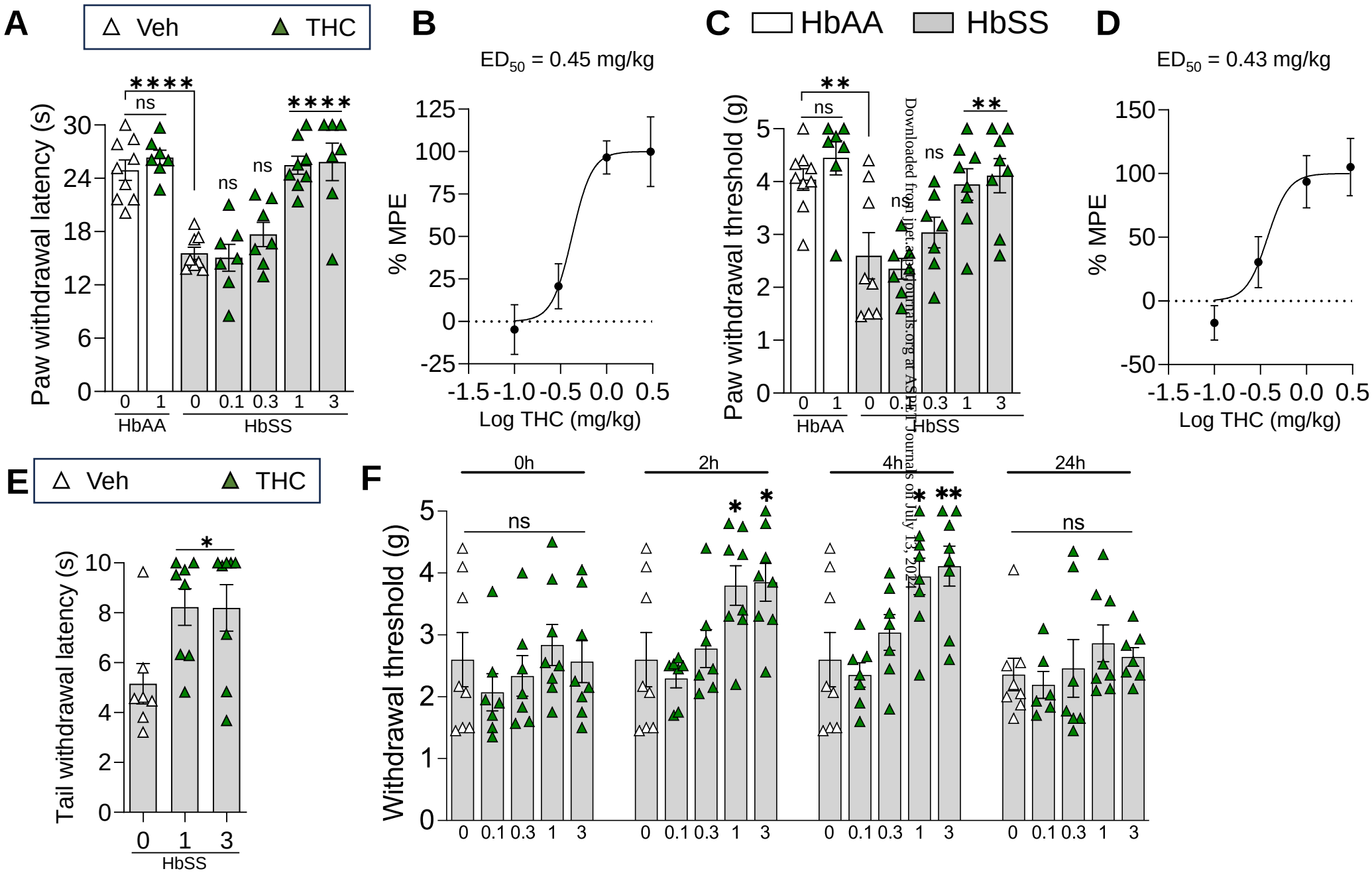

Figure 2

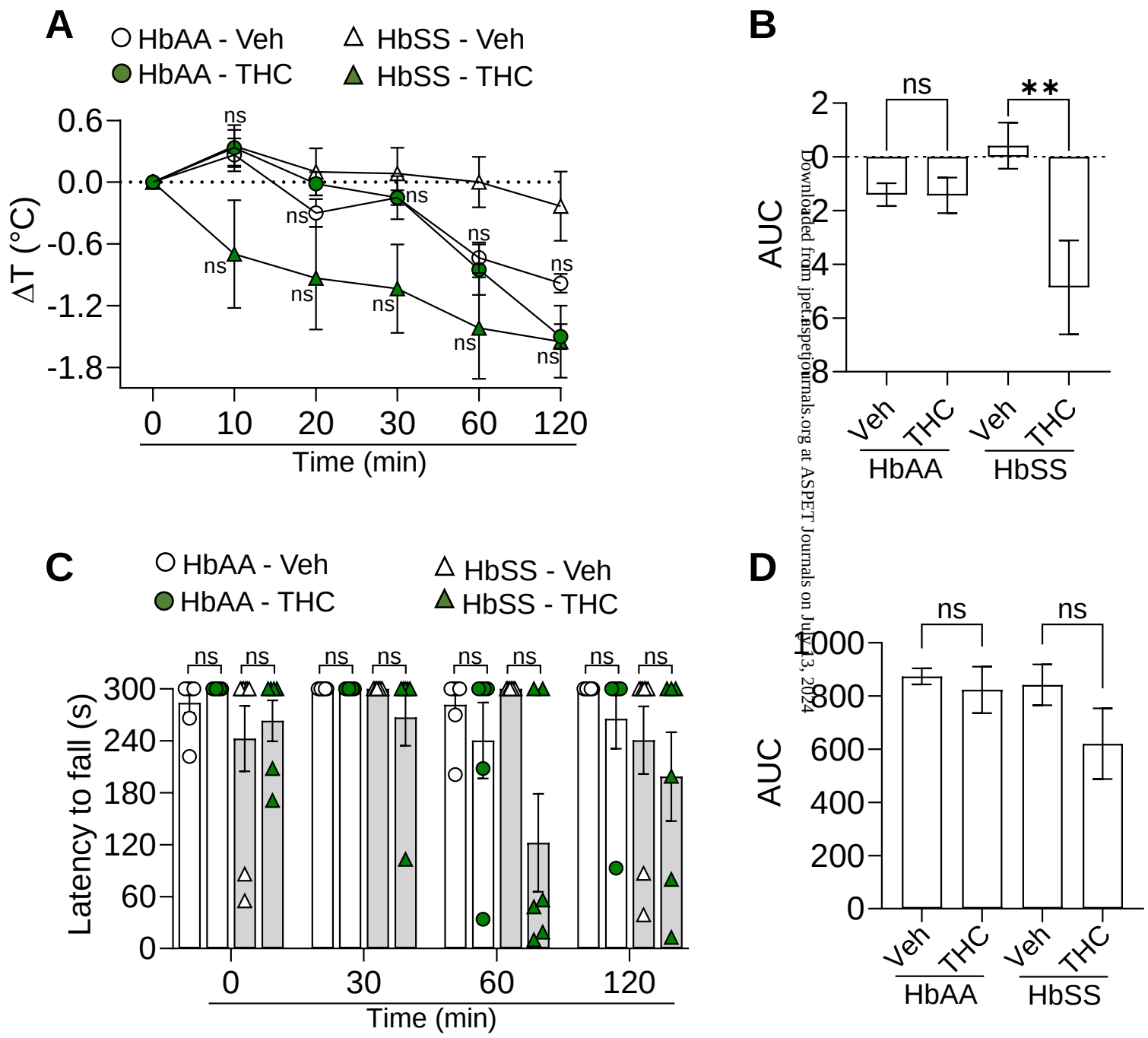

Figure 3

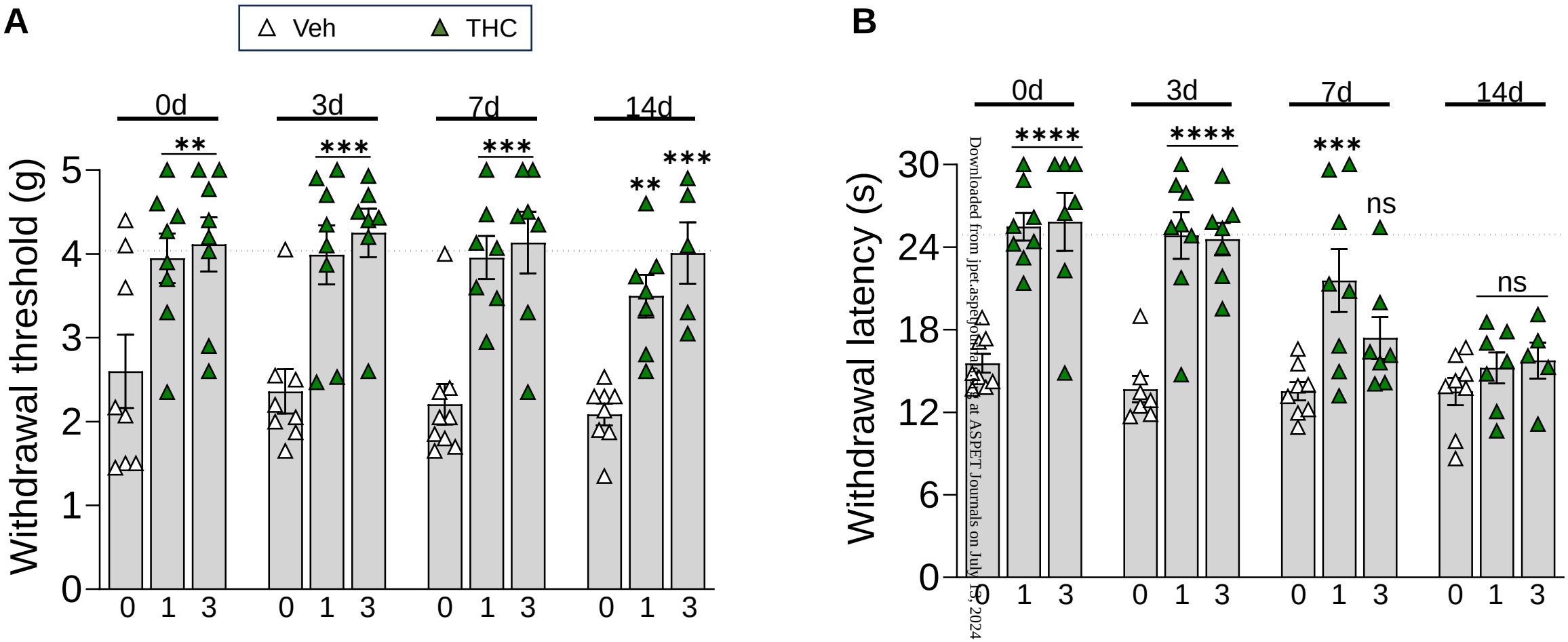

Figure 4