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Persistent Exposure to Δ^9-Tetrahydrocannabinol during Adolescence Does Not Affect Nociceptive Responding in Adult Mice

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ABSTRACT

Evidence suggests that Δ^9-tetrahydrocannabinol (Δ^9-THC), the intoxicating component of cannabis, causes enduring changes in the structure and function of adolescent brain circuits implicated in nociceptive responding. However, whether such changes might persistently disrupt nociceptive behaviors remains unknown. In the present study, we subjected C57BL6/J mice of both sexes to once-daily injections of Δ^9-THC (5 mg-kg^{-1}, i.p.) or vehicle throughout adolescence (PND 30–43) and, when the animals had reached adulthood (PND 70), assessed nociceptive behavior using the formalin and chronic constriction injury tests. We also investigated, using the tail immersion test, the antinociceptive effects of morphine and the development of tolerance to such effects. The results show that adolescent Δ^9-THC exposure does not significantly impair nociceptive responding or morphine-related antinociception and tolerance. The findings suggest that frequent exposure to a moderate dose of Δ^9-THC during adolescence does not permanently alter nociceptive circuits in male or female mice.

SIGNIFICANCE STATEMENT

The endocannabinoid system serves critical functions in the central and peripheral nervous systems, including regulation of pain, and can be modified by prolonged exposure to the intoxicating constituent of cannabis, Δ^9-tetrahydrocannabinol (Δ^9-THC). This raises the possibility that regular use of Δ^9-THC–containing cannabis during adolescence might cause changes in nociception that persist into adulthood. This study found that frequent early-life exposure to a moderate dose of Δ^9-THC does not permanently alter nociceptive function in male or female mice.

Introduction

The psychotropic constituent of cannabis, Δ^9-tetrahydrocannabinol (Δ^9-THC), binds to G-protein–coupled cannabinoid receptors, mimicking the actions of two endogenous lipid-derived transmitters, anandamide and 2-arachidonoyl-*sn*-glycerol (Lu and Mackie, 2016). These endocannabinoid messengers contribute to many physiologic and pathologic processes, including pain, energy metabolism, cognition, and mood (Piomelli, 2013; Finn et al., 2021; Van Egmond et al., 2021). Evidence indicates that the endocannabinoid system plays important roles in brain development and undergoes substantial changes during adolescence (Meyer et al., 2018), a time when many people start using cannabis and its derivatives (Johnston et al., 2017; Taylor et al., 2017). This chronological overlap is significant because an excessive use of cannabis during this critical period may interfere with endocannabinoid activity and might thus sway neural development in persistent or even permanent ways.

Previous work has shown that early-life exposure to Δ^9-THC causes persistent molecular, morphologic, and functional changes in the developing brain (Miller et al., 2019; Blest-Hopley et al., 2020). These include downregulation and desensitization of cannabinoid receptor subtype 1(CB_1) (Rubino et al., 2015), decreased availability of anandamide and 2-arachidonoyl-*sn*-glycerol (Rubino et al., 2015), loss of neural connectivity (Rubino et al., 2009; Ruiz et al., 2021; Zalesky et al., 2012), dysfunction of excitatory-inhibitory neurotransmitter balance (Prescot et al., 2013; Zamberletti et al., 2016; Renard et al., 2017), and increased dopaminergic neuronal activity (Renard et al., 2017). Several brain structures affected by early-life treatment with Δ^9-THC—e.g., medial prefrontal cortex, hippocampus, and thalamus—are implicated in nociceptive processing (Kuner and Kuner, 2021), yet the long-term consequences of adolescent Δ^9-THC exposure on pain-related behaviors remain unknown.

To fill this knowledge gap, we exposed male and female mice to once-daily injections of Δ^9-THC (5 mg-kg^{-1}, i.p.) or vehicle throughout adolescence [postnatal day (PND) 30–43] and, when they had reached adulthood (PND 70), assessed nociceptive behavior using the formalin and chronic

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ABBREVIATIONS: AUC, area under the curve; CCI, chronic constriction injury; CI, confidence interval; PFD, postformalin day; PND, postnatal day; POD, postoperative day; Δ^9-THC, Δ^9-tetrahydrocannabinol.

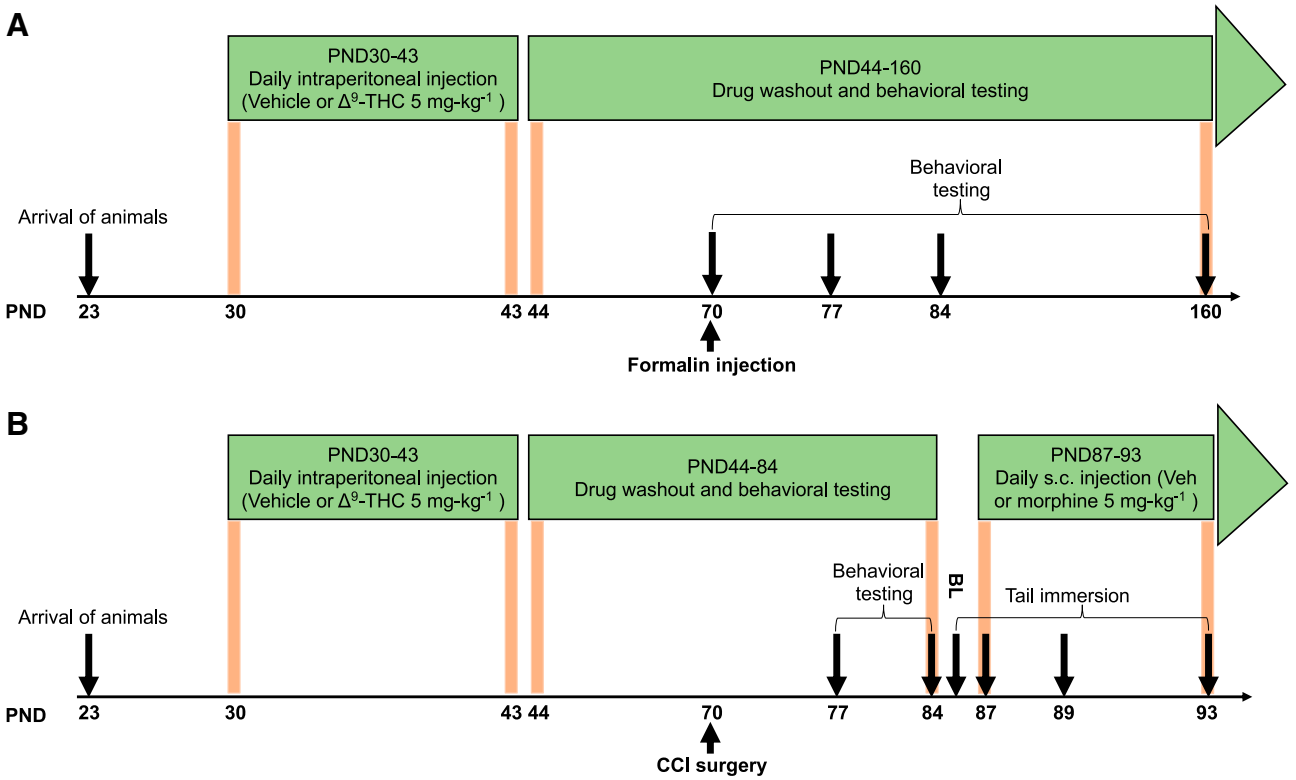

Fig. 1. Timeline of experimental procedure. Male and female mice received once-daily injections of Δ^9-THC or its vehicle (5 mg-kg^{-1}, i.p.) throughout adolescence (PND 30–43), followed by a home-cage washout period (PND 44–70) before intraplantar formalin injection (A) or CCI surgery (B). (A) The spontaneous pain response was assessed immediately after formalin injection. Mechanical allodynia, heat hyperalgesia, and paw edema were measured on PFD 7, 14, and 90. (B) CCI-evoked sensory abnormalities were assessed on POD 7 and 14 in both operated and nonoperated limbs. CCI mice were given morphine (5 mg-kg^{-1}, s.c.) twice daily for 7 days starting at POD 17, and nociceptive thresholds were monitored on days 1, 5, and 7 using the tail immersion test. Baseline tail withdrawal latencies were measured. BL, baseline.

constriction injury (CCI) tests, two widely used models of acute and persistent pain. The results show that adolescent Δ^9-THC exposure does not significantly alter nociceptive responding or the antinociceptive effects of morphine in these models.

Materials and Methods

Animals. Male and female C57BL/6J mice were from Charles River (Wilmington, MA) and arrived in the vivarium at PND 23. They were housed in single-sex groups of five per cage and were maintained on a 12-hour light/dark cycle (lights on from 6:30 to 18:30) in controlled temperature (20 ± 2°C) and relative humidity (55%–60%) with ad libitum access to food and water. All procedures were approved by the Institutional Animal Care and Use Committee at the University of California, Irvine, and were carried out in strict accordance with the National Institutes of Health guidelines for the care and use of animals.

Drug Preparation and Administration. Δ^9-THC (Cayman Chemicals, Ann Arbor, MI) was prepared shortly before experiments by evaporating the commercial acetonitrile solution under a light stream of N_2 and dissolving the residue in a vehicle of Tween 80/saline (5:95, vol). Starting at PND 30, mice were given 14 daily injections of Δ^9-THC or its vehicle (5 mg-kg^{-1}, i.p.) in 10 ml-kg^{-1} of body weight, followed by a home-cage washout period of 27 days. Morphine was prepared shortly before use by dissolving morphine sulfate (Sigma Aldrich, St. Louis, MO) in distilled water and was injected subcutaneously at 5 mg/kg twice daily for 7 days in a volume of 10 ml/kg. All mice were housed with cage mates receiving the same treatment.

Formalin Test. We injected formalin (0.3% or 1% vol, 20 μl) into the plantar surface of the right hind paw (PND 70). Following injection, the mice were immediately transferred to a transparent observation chamber, where nocifensive behavior was video recorded for 60 minutes. Mechanical allodynia, heat hyperalgesia, and edema were measured on postformalin day (PFD) 7, 14, and 90 in both injected and noninjected (contralateral) hind paws.

Chronic Constriction Injury. Adult mice (PND 70) were anesthetized with isoflurane, and the right common sciatic nerve was exposed at the level of the middle thigh by blunt dissection under aseptic conditions. Proximal to the trifurcation, the nerve was cleaned from surrounding connective tissue, and three chromic cat gut ligatures (4-0; Ethicon, Somerville) were loosely tied around it at 1-mm intervals. The wound was closed with a single muscle suture and skin clips. Operated mice were returned to their home cages for recovery. Mechanical allodynia and heat hyperalgesia were assessed on postoperative day (POD) 7 and 14 in both operated and nonoperated (contralateral) hind paws. The tail immersion test was performed starting at POD 15.

Tail Immersion. Tail immersion was performed as reported elsewhere (Fotio et al., 2020). Briefly, mice were gently handheld in a soft tissue pocket. The distal half of the tail was submerged in a water bath maintained at 54°C, and the latency to tail withdrawal was measured (in seconds). Tail withdrawal latencies were taken in duplicate (5-minute interval between trials) and averaged. Cutoff time was set at 10 seconds.

Behavioral Testing. All behavioral tests were carried out under blinded experimental conditions during the light cycle, as described previously (Mabou Tagne et al., 2021). In brief, nocifensive behavior was quantified from video recordings by a trained observer who was blinded to treatment. The nocifensive score is the sum of time spent

licking or biting the injected paw and the number of shakings. The area under the curve (AUC) was calculated for phase I (0–15 minutes) and phase II (15–50 minutes) using the trapezoidal rule. Mechanical allodynia was assessed using a dynamic plantar aesthesiometer (Ugo Basile, Italy) and was expressed as paw withdrawal threshold (in grams). Thermal hyperalgesia was measured using a Hargreaves plantar test apparatus (San Diego Instruments, CA) and was expressed as paw withdrawal latency (in seconds). Paw withdrawal measures were taken in duplicate with a 3-minute interval between stimuli and averaged. Paw swelling was measured using a digital caliper (Fisher Scientific) and is expressed as the difference in thickness (Δ thickness, millimeters) between injected and noninjected paws.

Data Analysis. Results are presented as means ± S.E.M of n experiments. Based on our previous work, $n = 8$ mice per group provides sufficient statistical power to detect differences across groups (Fotio et al., 2020; Mabou Tagne et al., 2021). Statistical analyses were conducted using GraphPad Prism version 9.1.0 (GraphPad Software, San Diego, CA). Differences between groups were determined by one-way or two-way ANOVA with Tukey or Šídák's post hoc test, as appropriate. The significance level was set at $P < 0.05$. Data were not transformed, and no data were excluded.

Results

Δ^9-THC Exposure Does Not Alter Nociceptive Behavior in the Formalin Model. We compared nociceptive responses to intraplantar formalin injection (0.3% or 1% vol) in adult (PND 70) male and female mice that had received throughout adolescence (PND 30–44) once-daily injections of Δ^9-THC (5 mg-kg^{-1}, i.p.) or its vehicle. As expected from previous work (Dubuisson and Dennis, 1977), formalin elicited—at both doses and in animals of both sexes—an immediate nocifensive reaction consisting of two temporally distinct phases of licking and flinching of the afflicted paw (phase I: 0–10 minutes; phase II: 15–60 minutes) (Figs. 2 and 3). At the 0.3% dose, formalin produced moderate paw edema in male and female mice, which disappeared within 14 days (Fig. 2, C and F). By contrast, 1% formalin caused profound swelling (Fig. 3, C and D), which lasted more than 2 weeks (Fig. 3, C and F) and was associated with persistent bilateral hypersensitivity to slightly painful heat stimuli (heat hyperalgesia) and normally innocuous mechanical stimuli (mechanical allodynia) (Fig. 4). We detected little or no statistical differences in these responses between vehicle- and Δ^9-THC–exposed mice of either sex (Figs. 2, 3, and 4).

Δ^9-THC Exposure Does Not Alter Persistent Nociceptive Behavior in the CCI Model. To determine whether early-life exposure to Δ^9-THC might affect nociception in a model of persistent neuropathic pain, we subjected vehicle- or Δ^9-THC–treated male mice to CCI and assessed their nociceptive responses 7 and 14 days later. In contralateral nonoperated paws, there was no statistically detectable difference between control and Δ^9-THC–exposed mice in withdrawal threshold (Δmean = −0.06 seconds; 95% CI : −0.7518 to 0.6318 seconds; $P = 0.9998$; Fig. 5A) or withdrawal latency (Δmean = −0.44 seconds; 95% CI: −1.828 to 0.9477 seconds; $P = 0.9351$; Fig. 5B). Similarly, in the operated paws, which developed mechanical allodynia (POD 7: Δmean = 3.144 seconds; 95% CI: 2.452–3.836 seconds; $P < 0.0001$; POD 14: Δmean = 3.236 seconds; 95% CI: 2.544–3.928 seconds; $P < 0.0001$; Fig. 5A) and heat hyperalgesia (POD 7: Δmean = 8.039 seconds; 95% CI: 6.651–9.427 seconds; $P < 0.0001$; POD 14: Δmean = 8.087 seconds; 95% CI: 6.699–9.475 seconds; $P < 0.0001$; Fig. 5B), nociceptive responses were statistically indistinguishable between control and Δ^9-THC–exposed animals (withdrawal thresholds: $F_{2,\ 54} = 0.4505$; $P = 0.6397$; withdrawal latencies: $F_{2,\ 54} = 2.427$; $P = 0.0979$).

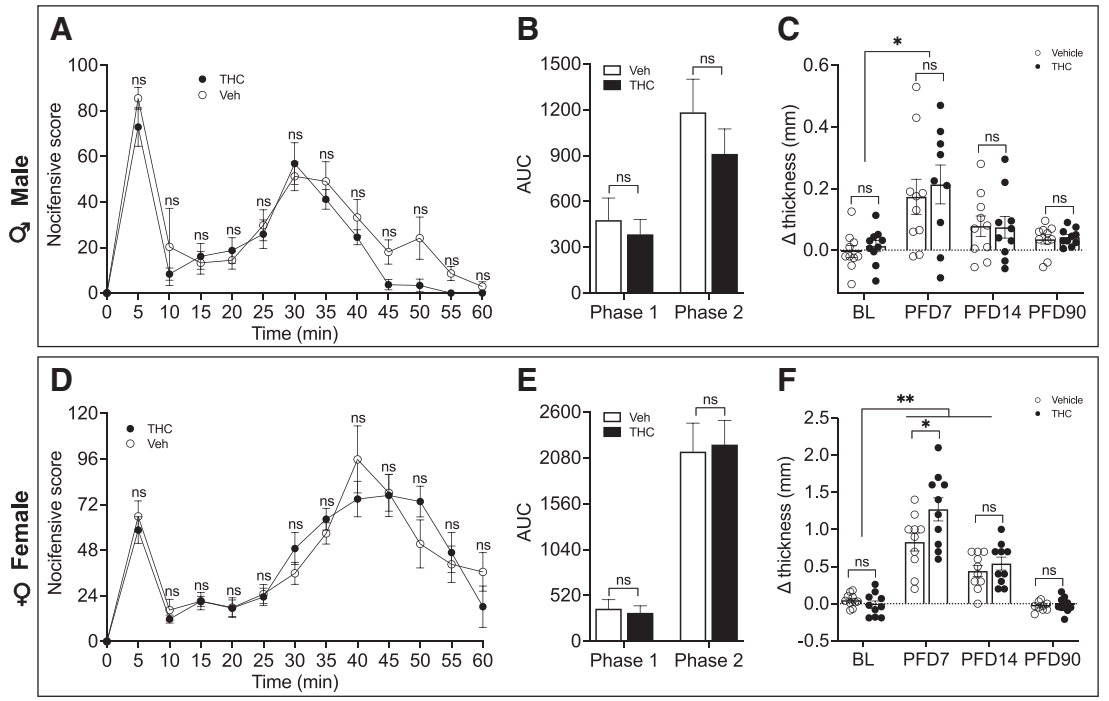

Fig. 2. Nociceptive and inflammatory responses to a low formalin dose (0.3%) in Δ^9-THC–exposed (closed symbols) or vehicle-exposed (open symbols) mice. (A and D) Time course of the nocifensive response in adult males ($F_{12,\ 216} = 0.8741$; $P = 0.5742$) and females ($F_{12,\ 216} = 1.150$; $P = 0.3212$). (B and E) AUC of nocifensive behavior for phase I (0–10 minutes) and phase II (15–60 minutes) in males ($F_{1,\ 230} = 0.1246$; $P = 0.7244$) and females ($F_{1,\ 230} = 0.02704$; $P = 0.8695$). (C and F) Paw edema (Δ thickness, millimeters) measured at PFD 7, 14, and 90 in males ($F_{3,\ 71} = 0.1399$; $P = 0.9358$) and females ($F_{3,\ 71} = 3.355$; $P = 0.0236$). Data are expressed as means ± S.E.M ($n = 9$ to 10 per group) and were analyzed by two-way ANOVA followed by Šídák's (A, B, D, and E) or Tukey's (C and F) multiple comparisons test. *$P < 0.05$ and **$P < 0.01$ vs. BL or vehicle controls. BL, baseline; ns, nonsignificant; Veh, vehicle.

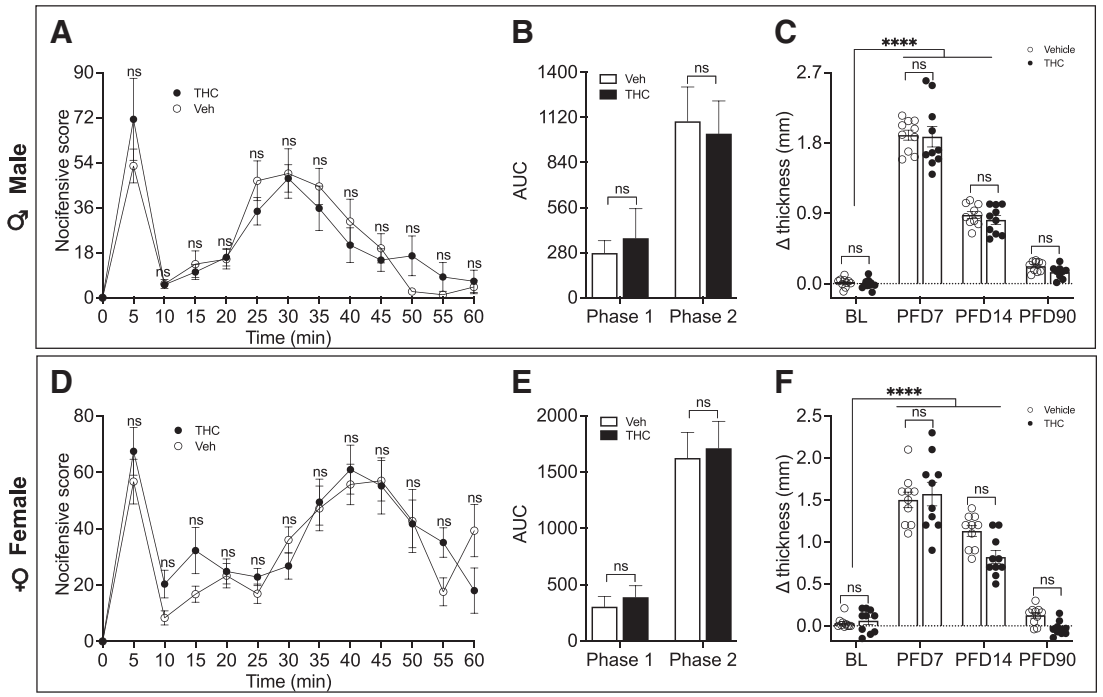

Fig. 3. Nociceptive and inflammatory responses to a high formalin dose (1%) in Δ^9-THC–exposed (closed symbols) or vehicle-exposed (open symbols) mice. (A and D) Time course of the nocifensive response in adult male ($F_{12,\ 216} = 1.030$; $P = 0.4227$) and female ($F_{12,\ 216} = 1.332$; $P = 0.2016$) mice. (B and E) AUC of nocifensive behavior for phase I (0–10 minutes) and phase II (15–60 minutes) in male ($F_{1,\ 230} = 0.09490$; $P = 0.7583$) and female ($F_{1,\ 230} = 0.00003$; $P = 0.9958$) mice. (C and F) Paw edema (Δ thickness, mm) measured at PFD 7, 14, and 90 in males ($F_{3,\ 71} = 0.1203$; $P = 0.9478$) and females ($F_{3,\ 71} = 2.922$; $P = 0.0397$). Data are expressed as means ± S.E.M (n = 9 to 10 per group) and were analyzed by two-way ANOVA followed by Šídák's (A, B, D, and E) or Tukey's (C and F) multiple comparisons test. ****$P < 0.0001$ vs. BL or vehicle controls. BL, baseline; ns, nonsignificant; Veh, vehicle.

Δ^9-THC Exposure Does Not Alter the Antinociceptive Response to Morphine. Finally, we asked whether adolescent exposure to Δ^9-THC might impact the antinociceptive effects of morphine or the development of tolerance to such effects. Adult CCI mice that had been exposed to Δ^9-THC during adolescence were given

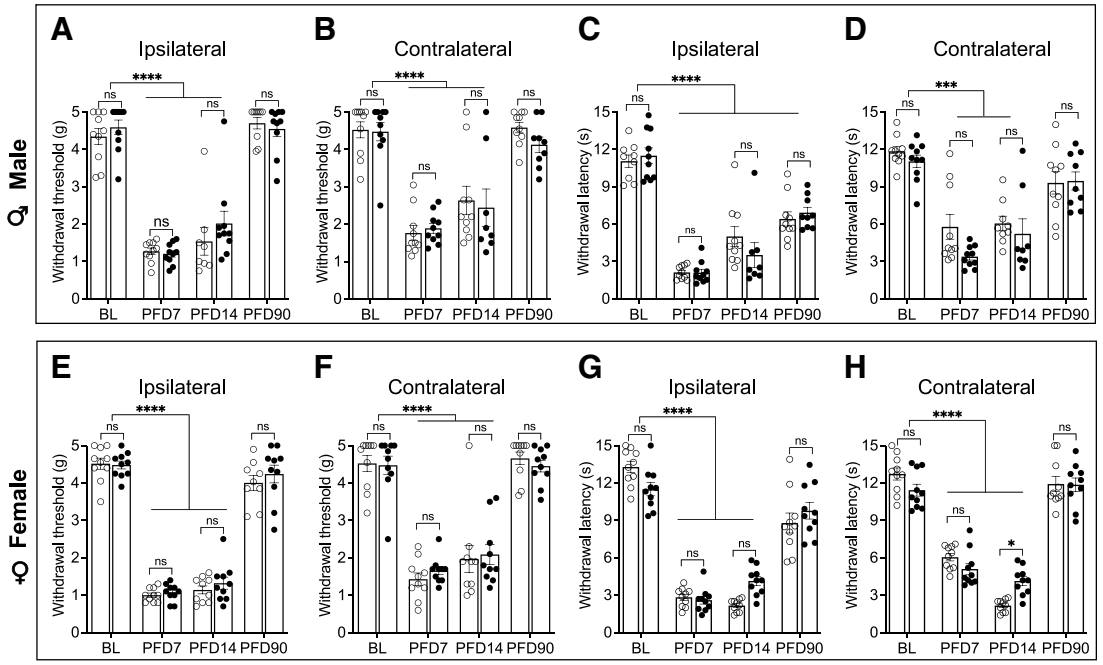

Fig. 4. Effects of 1% formalin on bilateral hyperalgesia and allodynia in Δ^9-THC–exposed (closed symbols) or vehicle-exposed (open symbols) mice of both sexes. (A, B, E, and F) Ipsilateral and contralateral mechanical allodynia. (C, D, G, and H) Ipsilateral and contralateral heat hyperalgesia. Data are expressed as means ± S.E.M (n = 8–10 per group) and were analyzed by two-way ANOVA with Tukey's post hoc test. *$P < 0.05$ and ****$P < 0.0001$ vs. BL or vehicle controls. Male: ipsilateral allodynia ($F_{3,\ 69} = 0.8588$; $P = 0.4667$) and hyperalgesia ($F_{3,\ 68} = 1.236$; $P = 0.3036$); contralateral allodynia ($F_{3,\ 69} = 0.4377$; $P = 0.7267$) and hyperalgesia ($F_{3,\ 68} = 1.071$; $P = 0.3673$). Female: ipsilateral allodynia ($F_{3,\ 71} = 0.3371$; $P = 0.7986$) and hyperalgesia ($F_{3,\ 72} = 5.616$; $P = 0.0016$); contralateral allodynia ($F_{3,\ 71} = 5.539$; $P = 0.0018$) and hyperalgesia ($F_{3,\ 72} = 5.539$; $P = 0.0018$). BL, baseline; ns, nonsignificant.

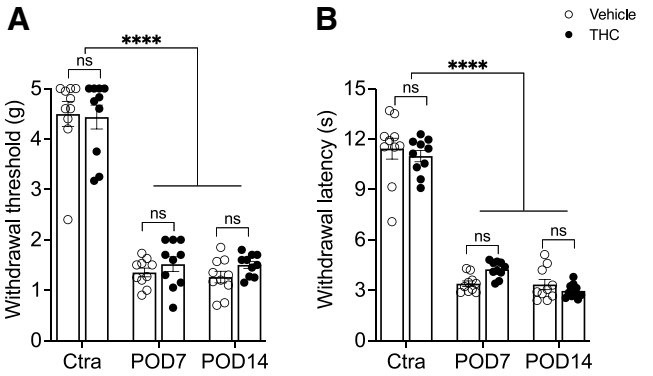

Fig. 5. Effects of CCI in Δ^9-THC–exposed (closed symbols) or vehicle-exposed (open symbols) male mice. (A) Mechanical allodynia and (B) heat hyperalgesia in operated paws 7 and 14 days after surgery (POD). Data are expressed as means ± S.E.M (n = 10 per group) and were analyzed by two-way ANOVA with Tukey's post hoc test. ****P < 0.0001 vs. Ctra. Ctra, contralateral; ns, nonsignificant.

morphine (5 mg-kg^{-1}, s.c.) twice daily for 7 days, and their nociceptive thresholds were monitored on days 1, 5, and 7 using the tail immersion test. Baseline tail withdrawal latencies in control mice (Fig. 6A) and Δ^9-THC–exposed mice (Fig. 6B) prior to treatment with morphine were (mean ± S.D.) 1.79 ± 0.34 seconds and 1.68 ± 0.31 seconds, respectively. Morphine produced marked antinociception on test day 1 (vehicle: Δmean = −6.976 seconds, 95% CI: −9.245 to −4.707, P < 0.0001; Δ^9-THC: Δmean = −8.321 seconds; 95% CI: −10.06 to −6.586; P < 0.0001), which gradually waned over the following days until it became statistically undetectable on day 7 (vehicle: Δmean = −0.7010 seconds, 95% CI: −2.970 to 1.568, P = 0.7903; Δ^9-THC: Δmean = −0.6945, 95% CI: −2.429 to 1.040, P = 0.6416). There was no statistically detectable difference in withdrawal latency between Δ^9-THC– and vehicle-exposed mice ($F_{2,\ 54}$ = 0.7732; P = 0.4666) (Fig. 6C).

Discussion

There is growing concern that cannabis legalization will increase access to the drug for young people, although no available data support this possibility (Rotermann, 2019; Hammond et al., 2020; Miech et al., 2020; Paschall et al., 2021). Δ^9-THC, the drug's main intoxicating component, may persistently impact endocannabinoid signaling (Blest-Hopley et al., 2020) and may cause enduring changes in the structure and function of adolescent brain circuits implicated in nociceptive responding (Rubino et al., 2009, 2015; Zalesky et al., 2012; Prescot et al., 2013; Zamberletti et al., 2016; Renard et al., 2017). However, it remains to be determined whether such changes might persistently affect nociceptive behaviors. The present study provides the first evidence to suggest that daily administration of Δ^9-THC in adolescent mice of both sexes—at a dose (5 mg/kg) that produces moderate but pharmacologically relevant exposure to the drug (Torrens et al., 2020)—does not impair nociception in adulthood.

At the start of this study, we hypothesized that adult mice that had been exposed to Δ^9-THC during adolescence might have lower than normal nociceptive thresholds, which might make them more sensitive to acute and chronic pain. This idea was supported by two lines of evidence. First, prior studies have found that administration of Δ^9-THC during adolescence produces substantial alterations in nociceptive brain circuitry that extend into adulthood (Zalesky et al., 2012; Prescot et al., 2013; Zamberletti et al., 2016; Renard et al., 2017). Second, adolescent Δ^9-THC affects endocannabinoid signaling in brain regions that are implicated in pain regulation (Ellgren et al., 2008; Rubino et al., 2015). We also speculated that adolescent Δ^9-THC might persistently alter the antinociceptive effects of morphine and the development of tolerance to such effects. This hypothesis was consistent with the observation that adolescent exposure to the drug produces discrete changes in opioid peptide levels and opioid receptor numbers in brain areas implicated in the control of pain (Ellgren et al., 2007, 2008).

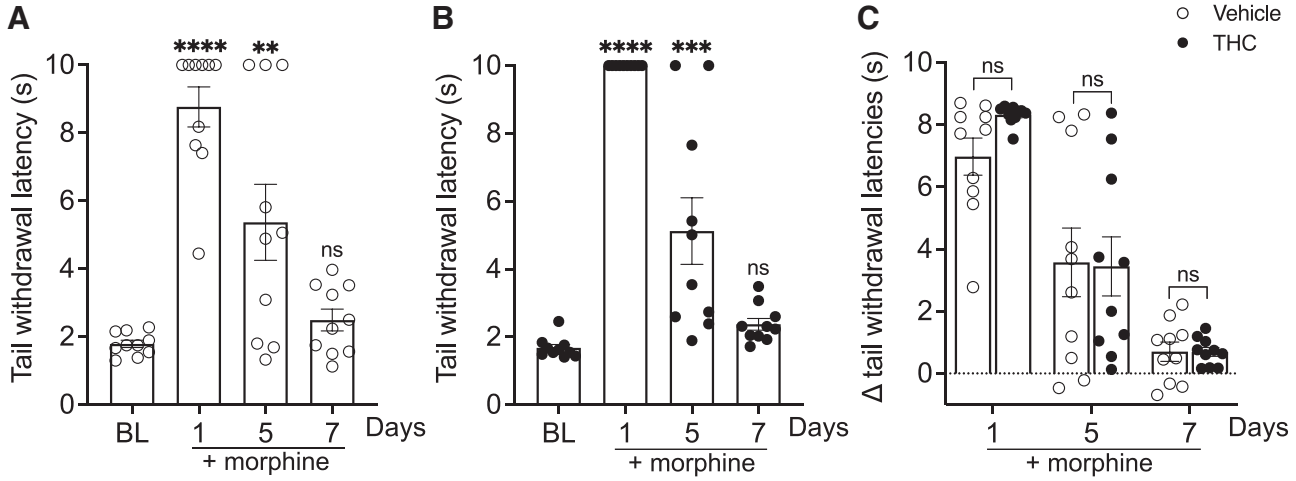

Fig. 6. Antinociceptive effects of morphine in Δ^9-THC–exposed (closed symbols) or vehicle-exposed (open symbols) male mice subjected to CCI. Tail withdrawal thresholds were measured prior to (BL) and 30 minutes after treatment with morphine (5 mg-kg^{-1}, s.c.) on days 1, 5, and 7. (A) Tail withdrawal latency (in seconds) in vehicle control animals (open circles). (B) Tail withdrawal latency (in seconds) in Δ^9-THC–exposed animals (closed circles). (C) Δ tail withdrawal latencies (in seconds) in Δ^9-THC–exposed mice and animal controls. Data are expressed as means ± S.E.M (n = 10 per group) and were analyzed by one-way (A and B) or two-way (C) ANOVA with Dunnett's (A and B) or Šídák's (C) post hoc test. **P < 0.01, ***P < 0.001, and ****P < 0.0001 vs. BL or vehicle controls. BL, baseline; ns, nonsignificant.

To assess the long-term impact of adolescent Δ^9-THC exposure on nociceptive responding in mice, we used intraplantar formalin injection and CCI of the sciatic nerve, two models that capture acute and persistent aspects of injury-induced pain, respectively (Gregory et al., 2013). We implemented the CCI model as previously described (Bennett and Xie, 1988) and injected formalin at two concentrations (0.3% and 1% vol). At 0.3%, formalin evoked the expected behavioral response but only moderate and short-lived local tissue inflammation (paw edema), which was not accompanied by persistent hypersensitivity (Figs. 2 and 3). On the other hand, the nociceptive reaction to 1% formalin was accompanied by both profound paw edema and bilateral sensory hypersensitivity, which lasted for more than 2 weeks (Figs. 2, 3, and 4). The graded response to formalin was exploited to assess whether mice that had received Δ^9-THC in adolescence might be more or less vulnerable to painful stimuli.

Although the Δ^9-THC treatment regimen used here was shown to yield pharmacologically relevant exposure to the drug (Torrens et al., 2020) and to affect behavior both acutely (Ruiz et al., 2020) and permanently (Schoch et al., 2018), we found that it had no detectable effect on either pain behaviors produced by formalin and CCI or morphine-related antinociception and tolerance. We conclude that frequent exposure to a moderate dose of Δ^9-THC during adolescence does not permanently alter nociceptive circuits in male and female mice. It is possible, although it remains to be demonstrated, that this lack of effect might in fact result from neurodevelopmental compensation—despite persistent deficits—in nociceptive circuits of the central and peripheral nervous systems. Of note, even though sex-dependent differences have been demonstrated in some persistent effects of adolescent Δ^9-THC administration (Rubino and Parolaro, 2015; Silva et al., 2015; Schoch et al., 2018), we found no such difference in the response to formalin.

Some limitations in the external validity of this study should be noted. First, our Δ^9-THC exposure protocol captures only one of the many possible modalities of cannabis use, which in the real world vary greatly in dosage, frequency, and route selection (Taylor et al., 2017; Hammond et al., 2020). Second, the use of Δ^9-THC alone and at a relatively moderate dosage (Torrens et al., 2020) does not match many cannabis products currently available to the public, which may deliver high doses of Δ^9-THC or may contain other chemical constituents that interact functionally with Δ^9-THC (e.g., cannabidiol) (Daniulaityte et al., 2017). Lastly, we used only two animal models of pain, which do not fully recapitulate the human pain experience.

Despite these limitations, the present study shows that daily exposure to an ecologically relevant dose of Δ^9-THC throughout adolescence does not affect nociceptive responding in adult mice. The data do not allow us to determine whether neurodevelopmental compensation partially or completely underpins this negative result.

Authorship Contributions

Participated in research design: Mabou Tagne, Fotio, Piomelli.

Conducted experiments: Mabou Tagne, Fotio, Ibne Rashid.

Performed data analysis: Mabou Tagne.

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

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