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Age-Related Differences in Δ^9-Tetrahydrocannabinol-Induced Antinociception in Female and Male Rats

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Given the use of cannabis as an analgesic by a broadening age range of patients, the aim of this study was to determine whether the antinociceptive effects of Δ^9-tetrahydrocannabinol (THC) differ by age. The antinociceptive potency and efficacy of THC (1.0–18 mg/kg ip) was compared in male and female rats aged postnatal day 35–40 (adolescent), 60–70 (young adult), and 291–325 (middle-aged adult), using warm water tail withdrawal and paw pressure tests. Motoric effects of THC were assessed using a locomotor activity test. On the tail withdrawal test, THC was significantly more effective in middle-aged adult than in young adult rats and significantly less effective in adolescent than in young adult rats. Similar but smaller age-related differences were observed on the paw pressure test. Sex differences in THC's antinociceptive effects were consistent across the 3 ages examined, with greater THC effects observed in females than males of each age. Age-related differences in THC's locomotor-suppressing effect were also observed, with the greatest effect in young adult female rats. Serum THC levels were slightly higher in adolescent than in young adult rats, and levels of the active metabolites 11-OH-THC and cannabinol, as well as the inactive metabolite 11-nor-9-carboxy-THC, did not differ between adolescent and young adult rats. These results suggest that the pain-relieving effects of THC may be more limited in adolescents than in adults and that these age-related differences in THC effect are not attributable to differential absorption or metabolism of THC.

Public Health Significance
This study demonstrates age-related differences in sensitivity to the pain-relieving effects of the major psychoactive constituent of cannabis, Δ^9-tetrahydrocannabinol, and suggests that cannabis and related drugs may be less effective analgesics in adolescents than in adults.

Keywords: cannabinoid, analgesia, adolescents, development, sex differences

Pain relief is the most common reason given for medical cannabis use, and the age range of medical cannabis users is broadening (Haug et al., 2017; Sexton, Cuttler, Finnell, & Mischley, 2016; Walsh et al., 2013). To our knowledge, no studies have been conducted to compare cannabinoid analgesia in adolescent versus adult pain patients, or in younger versus older adult pain patients, or in different ages of healthy human participants subjected to pain in a laboratory setting. Even animal studies documenting the antinociceptive effects of cannabinoids have focused predominantly on young adult rats and mice, with rare exceptions. For example, Wiley, O'Connell, Tokarz, and Wright (2007) compared the acute antinociceptive effects of THC in early adolescent (postnatal day [PND] 29) versus young adult (PND 68) rats and found greater THC potency in female adults than adolescents, whereas THC potency in males did not differ significantly between adolescent and young adults.

The endocannabinoid system is known to change substantially from birth to adulthood. For example, de Fonseca, Ramos, Bonnin, and Fernández-Ruiz (1993) quantified cannabinoid Type I receptor (CB1R) density in limbic forebrain, striatum, and midbrain from the early neonatal period (PND 2–5) through adolescence and adulthood in male and female rats and reported a steady increase in CB1R density in both sexes until PND 30–40, after which density remained relatively stable or declined somewhat in all regions. A subsequent study documented similar increases in rat brain CB1R density from PND 7 to 60; increases were most marked in cerebellum but were observed in all six brain areas examined (Belue, Howlett, Westlake, & Hutchings, 1995). Additionally, several studies have demonstrated developmental fluctuations in brain levels of endogenous cannabinoids such as anand-

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amide and 2-arachidonoylglycerol (for a review, see Lee, Hill, & Lee, 2016). Systemically administered cannabinoids produce acute antinociception by acting at multiple sites from the periphery to spinal cord to brain (Starowicz & Finn, 2017), the latter specifically including the amygdala, periaqueductal gray (PAG), and rostral ventromedial medulla (RVM; Lichtman, Cook, & Martin, 1996; Martin et al., 1999; Meng, Manning, Martin, & Fields, 1998). Given that developmental changes in the endocannabinoid system have been demonstrated in brain areas that include these structures, it is likely that there are age-related differences in the pain-relieving effects of cannabinoids.

To test this hypothesis, we compared the antinociceptive effects of THC in adolescent rats (PND 35–40 [mid- to late adolescence]; Spear, 2000) versus adult rats of two different ages: young adults, at an age that is typical in rodent studies (PND 60–70), and middle-aged adults (PND 291–325), which are more representative of the developmental period of many chronic pain patients (Gibson & Lussier, 2012) but are considerably older than rodents typically tested in preclinical studies (Jackson et al., 2017). Both male and female rats were included. Given previous research demonstrating sex differences in cannabinoid antinociception in adolescent and young adult rats (for a review, see Cooper & Craft, 2018), we predicted that THC would be more potent in females than males at both younger ages. Given the lack of data comparing cannabinoid antinociception in older male versus female adults of any species, we made no sex difference predictions for that age; however, data from clinical trials indicate that cannabinoids are analgesic in middle-aged humans (e.g., Hoggart et al., 2015; Toth et al., 2012), and thus we predicted that THC would produce antinociception in middle-aged rats.

Method

Subjects

Male ($n = 81$) and female ($n = 76$) Sprague–Dawley rats within three age ranges served as subjects: PND 35–40 (average = 37 days old, both sexes), PND 60–70 (average = 65 ± 1 day old [males], 64 ± 1 day old [females], and PND 291–325 (average 306 ± 2 days old [males], 308 ± 2 days old [females]). Body weight in each group ranged as follows: adolescent males, 127–191 g; adolescent females, 78–150 g; young adult males, 253–386 g; young adult females, 171–267 g; middle-aged males, 449–557 g; middle-aged females, 258–352 g. Rats were bred in-house from Harlan stock (Envigo, Livermore, CA) and housed in same-sex and same-age pairs. Males and females were housed in the same vivarium room but on different racks. The vivarium was kept at 21 ± 2 °C and 20%–30% relative humidity, and rats were housed under a 12:12 light:dark cycle (lights on at 0700 hr). Rats were tested during daylight hours, typically between 1200 hr and 1500 hr. Rats had ad libitum access to food (Rodent Diet 5001; Animal Specialties, Woodburn, OR) and water at all times except during testing. All experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011), and protocols were approved by the Washington State University Institutional Animal Care and Use Committee.

Apparatus

Tail withdrawal testing was conducted using a 2.5-L water bath (Precision Scientific, Winchester, VA) heated to 50 ± .5 °C. Paw pressure testing was conducted using a rat Analgesy-meter (Ugo-Basile, Varese, Italy). For adults, two counterweights were used on the paw pressure apparatus, so that pressure increased at a constant rate of 48 g/s to a maximum of 720 g (=15-s cutoff). To adjust for adolescent rats' smaller size compared to adults, we used only one counterweight so that pressure increased at a constant rate of 32 g/s to a maximum of 480 g (=15-s cutoff). Horizontal activity was assessed using a photobeam apparatus (Columbus Instruments, Columbus, OH), which has 15 photobeams spaced 2.5 cm apart and 8 cm above the cage floor. When adolescents were tested, the photobeam emitter and detector panels were adjusted downward by 1.3 cm, to ensure that horizontal locomotor activity would be captured in these smaller rats.

Drug

THC was obtained from the National Institute on Drug Abuse Drug Supply Program (Bethesda, MD). The vehicle was 1:1:18 parts ethanol:cremaphor:saline.

Behavioral Procedures

Within each age group, rats were randomly assigned to either the vehicle control condition or THC condition. Experimenters were not blinded to treatment group assignment: The substantial size differences (and testes, in males) between age groups and sexes were visually apparent, as were behavioral differences once several doses of THC had been administered, in most rats. Three baseline trials were conducted on the tail withdrawal test and immediately thereafter the paw pressure test; each of the three sets of baseline trials was conducted approximately 5 min apart. For the tail withdrawal test, each rat was wrapped in a small cloth and the distal 5 cm (4 cm, for adolescents) of its tail was lowered into the heated water bath; latency in seconds to move the tail away from the water was recorded with a handheld stopwatch. A cutoff of 15 s was imposed to prevent tissue damage. For the paw pressure test, each rat was wrapped in a small cloth and the right hind paw was placed on the pedestal. A blunt probe was gently lowered onto the dorsal hind paw, and then electronic (foot pedal) activation of the apparatus started the steady pressure increase on the hind paw. Latency in seconds to withdraw or attempt to withdraw the paw from under the probe was recorded; a cutoff of 15 s was imposed to prevent tissue damage. After the third set of baseline trials, THC was injected intraperitoneally cumulatively, starting at 1.0 mg/kg and increasing to a total dose of 18 mg/kg (absolute doses of 1.0, .8, 1.4, 2.4, 4.4, 8.0 mg/kg were injected); 13 min after each injection, rats were tested on the tail withdrawal test, the paw pressure test, and a 5-min locomotor activity test, in that order. Rats in the vehicle control group were injected at the same intervals with vehicle only and then tested on all the same tests as the THC-treated rats were.

Serum Cannabinoid Analysis

Upon completion of behavioral testing, a subset of adolescent and young adult rats were euthanized via rapid decapitation, and

trunk blood was collected. Trunk blood samples were centrifuged for 20 min at 2000 g at 4 °C; serum was removed and stored at −80 °C until analysis. Concentrations of THC, 11-OH-THC, 11-nor-9-carboxy-THC (THC-COOH), and cannabinol (CBN) were determined to assess age-related differences in circulating THC and metabolites.

Quantitation of cannabinoids was achieved using an ultraperformance liquid chromatography system (Waters Acquity I-Class UPLC, Milford, MA) coupled with a quadrupole time of flight mass spectrometer (QTOF; Waters Xevo G2, Manchester, United Kingdom). The first step of sample preparation was sample centrifugation at 8,000 rpm for 10 min to remove any remaining cells. Then 180 uL of the resulting supernatant was spiked with 20 uL of solution containing 200 ppb each of the deuterated standards (THC-d3, OH-THC-d3, COOH-THC-d3, and CBN-d3; Cerilliant, Round Rock, TX). Combined with the high resolution and accurate mass of the QTOF platform, these internal standards can minimize contributions from nonideal metabolite extraction and instrumental variability and allow for direct quantitation of each targeted analyte. Following the internal standard addition, protein precipitation was promoted by adding 400 uL of cold acetonitrile (ACN) dropwise while vortexing. Immediately the samples were centrifuged at 10,000 g for 5.5 min at 25 °C; then supernatant was added to .65 mL of 1% ammonium hydroxide and vortexed before solid phase extraction (SPE). A mixed-mode SPE cartridge (OAXIS Max 1 cc, Waters, Ireland) was used for cannabinoid isolation. Each SPE cartridge was conditioned with 1 mL methanol followed by 1 mL 1% ammonium hydroxide. After the cartridge conditioning, the newly prepared sample was loaded onto the SPE cartridge and pulled through the system using a light vacuum (~1–2 psi). Then .5 mL of 35% ACN was added and allowed to dry under full vacuum for 10 min, and 1.5 mL of a hexane/ethyl acetate/acetic acid (49:49:2, vol/vol/v) mixture was used to elute the samples. The eluent was then evaporated under nitrogen at room temperature, and 130 uL of a methanol:water solution (80:20, vol/vol) was used for the final sample and transferred to an autosampler vial. Analyte separation was achieved using a 50-mm C18 BEH UPLC column (Waters, Milford, MA) kept at 40 °C. The mobile phases were high-purity water (Fisher Scientific Co., Fair Lawn, NJ) with .1% formic acid (A) and pure acetonitrile (Fisher Scientific Co., Fair Lawn, NJ) with .1% formic acid (B), respectively. Initially, mobile phase B was increased from 5% to 60% in .2 min and kept increasing to 90% at 3.5 min. This level was held for an additional .5 min. At 4 min, mobile B was decreased to its initial condition of 5% within .1 min and was held static for .9 min for column reequilibration. With an operational flow rate of .3 mL/min, a total of 10 uL of each prepared sample was injected onto the column. The obtained experimental data were analyzed by TargetLynx (Waters, Milford, MA) software, used to generate quantitative results. Briefly, the parameters used for TargetLynx were as follows: retention time window: ±.2 min, response use: integrated area, polynomial type: linear, and weighting function: 1/X.

Data Analysis

Baseline scores for each age group (within sex) on each nociceptive test were screened for outliers: Any individual trial on which latency to respond was greater than 3 standard deviations away from the mean of all values was dropped from the data set. Five tail withdrawal scores met this criterion (one from a male in the adolescent group, one from a female in the young adult group, one each from a male and two females in the middle-aged group) totaling ~1% of all baseline tail withdrawal scores. Nine paw pressure scores also met this criterion (one from a male in the adolescent group, one each from two males and one female in the young adult group, and one each from two males and three females in the middle-aged group), totaling ~2% of all baseline paw pressure scores. For each rat, the mean baseline score (based on three scores, for 94% of rats) was calculated for tail withdrawal and paw pressure tests. These mean baseline nociceptive latencies were then compared among age groups and between sexes by two-way analysis of variance (ANOVA). Locomotor activity data (number of photobeams broken in each 5-min test) were compared in control rats—those injected with vehicle only—among age groups and between sexes by three-way ANOVA, with variables of age (three levels), sex (two levels), and time (six levels, repeated).

Because there were significant group differences in nociceptive and locomotor baselines (see the Results section), data for each THC-treated rat were adjusted relative to its own baseline score (for nociceptive tests) or to the mean, same-age/same-sex control group locomotor score at each time point (for locomotor activity test) before analysis of THC effects. Specifically, percent maximum possible effect (%MPE) was calculated from each rat's THC tail withdrawal and paw pressure data as follows: (response latency after THC − baseline response latency)/(15 s − baseline response latency) × 100. Locomotor activity scores for each drug-treated rat were transformed to percent of control as follows: (number of photobeam breaks after THC/mean number of photobeam breaks in same, age/same-sex vehicle-treated rats) × 100. Percent MPE and percent of control locomotor data were then analyzed by ANOVA to determine whether there were age- and sex-related differences in THC effect. Significant age differences were followed by Tukey post hoc tests to determine which age groups differed from each other, and significant sex differences were followed by Student's *t* test with a Bonferroni correction to determine whether sex differences at each dose were significant within each age group ($ps \leq .05$ were considered statistically significant).

Because drug-induced decreases in general activity can contribute to delayed responses on the tail withdrawal and paw pressure tests, a Pearson correlation analysis was conducted on data within each age and sex to test the strength of the association between %MPE scores on each nociceptive test and percent of control locomotor scores.

Results

Nociceptive and Locomotor Baselines

Figure 1 (top panels) shows baseline tail withdrawal and paw pressure latencies in male and female rats at each of the three ages tested. Baseline tail withdrawal latencies were significantly longer in adult compared to adolescent rats: age, $F(2, 145) = 13.24$, $p < .001$, with no significant sex differences. Baseline paw pressure latencies also differed among the three age groups: They were somewhat longer in adolescent and middle-aged rats than in young adult rats: age, $F(2, 145) = 6.87$, $p = .001$. Paw pressure latencies

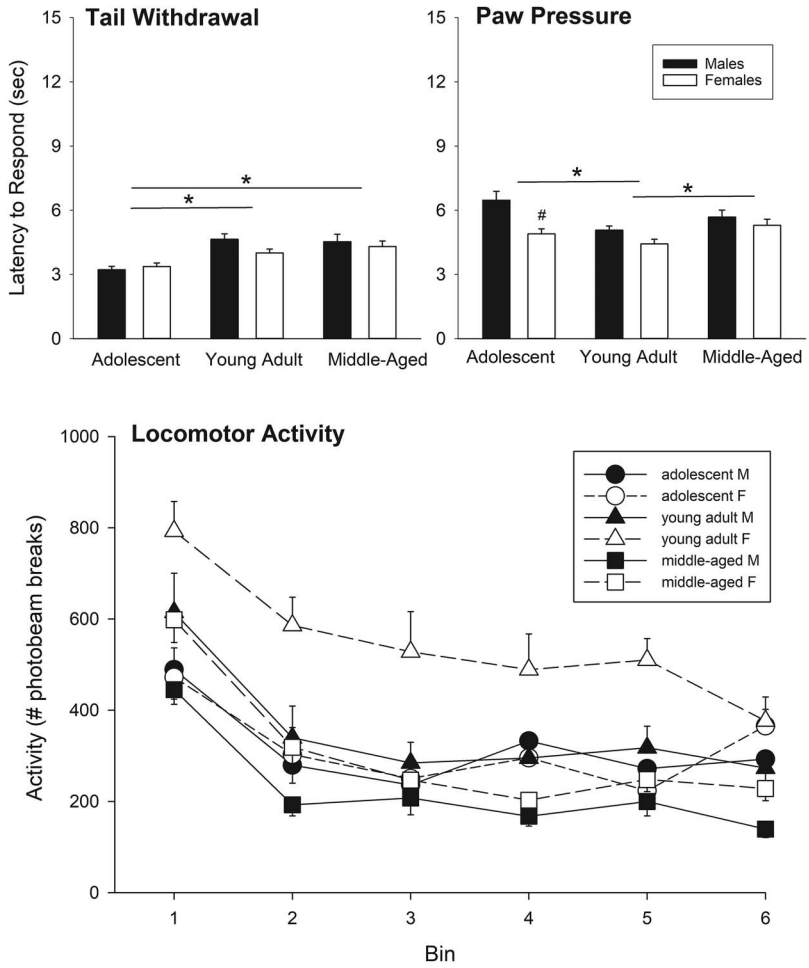

Figure 1. Top panels: Baseline response latencies on the 50 °C warm water tail withdrawal and paw pressure tests (n = 24–26 females and 26–29 males per age group; includes rats assigned to vehicle control and to Δ^9-tetrahydrocannabinol groups). Bottom panel: Locomotor activity in vehicle-treated control rats (n = 10 females and 10–13 males per age group); rats were injected with saline and 13 min later tested on tail withdrawal and paw pressure tests and then placed into locomotor activity chambers for 5 min—thus, bins were approximately 20 min apart. Top panels: Asterisks indicate a significant age difference; the number sign indicates a significant sex difference ($p \leq .05$). Bottom panel: Significant age and sex differences. Error bars indicate standard error of the means. M = male; F = female.

also were longer in males than in females: sex, $F(1, 145) = 14.06$, $p < .001$; post hoc comparisons within each age indicated that the sex difference was significant in adolescent rats only ($p = .001$). Within each age group, there were no significant differences in baseline nociceptive latencies between rats assigned to the vehicle control versus THC conditions (data are not shown).

Figure 1 (bottom panel) shows locomotor activity in vehicle-injected controls of each age and sex. Young adult rats were more active than adolescent and middle-aged rats were, and this age difference was particularly large in females: Age × Sex, $F(2, 57) = 4.32$, $p = .018$. When data within each sex were analyzed, young adult females were found to be significantly more active than were adolescent females ($p = .001$) or middle-aged females ($p < .001$), with no significant difference in activity between adolescent and middle-aged females. Among males, both adolescent ($p = .048$) and young adult ($p = .004$) rats were significantly more active than middle-aged adult rats were, with no significant difference between adolescent and middle-aged males. Regarding sex differences, females were more active than males were in the young adult ($p = .007$) and middle-aged ($p = .021$) adult groups but not in the adolescent group.

THC-Induced Antinociception

As shown in Figure 2, THC produced dose-dependent increases in %MPE on the tail withdrawal test, but efficacy differed among ages and between sexes. THC's antinociceptive effect was greatest in middle-aged adult rats, followed by young adult rats, with little to no effect observed in adolescent rats: Age × THC Dose, $F(10, 440) = 6.84$, $p < .001$. Post hoc tests indicated that THC effects were significantly greater in middle-aged adult than in young adult rats ($p = .027$) and greater in young adult than in adolescent rats

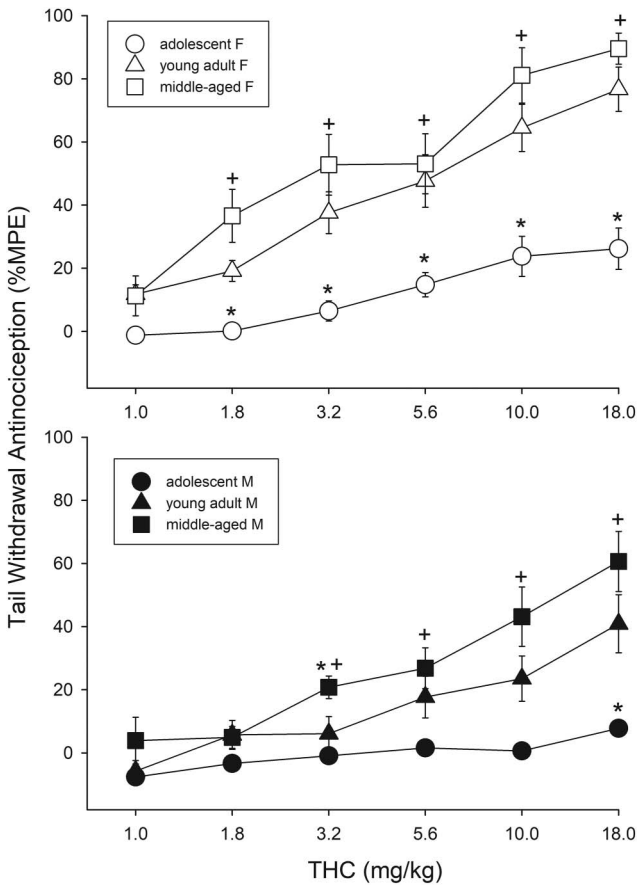

Figure 2. Age-dependent Δ^9-tetrahydrocannabinol (THC)-induced antinociception on the 50 °C warm water tail withdrawal test in female rats (top panel; $n = 14–16$ per age group) and in male rats (bottom panel; $n = 16$ per age group). Asterisks indicate significantly different from the young adult group at the same dose; plus signs indicate significantly different from the adolescent group at the same dose (Tukey post hoc test, $p < .05$). Error bars indicate standard error of the means. F = female; M = male.

($p < .001$). Regarding sex differences, THC produced greater effects in females than in males: Sex × THC Dose, $F(5, 440) = 4.47$, $p = .001$; this sex difference was similar at each age (no Sex × Age or Sex × Age × THC Dose interactions). Sex differences in maximal tail withdrawal effect are shown in Table 1.

Figure 3 shows that on the paw pressure test, age-related differences in THC effect were more modest, although still statistically significant: age, $F(2, 88) = 5.16$, $p = .008$, with effects in middle-aged adult rats greater than those in adolescent rats ($p = .009$) but no difference between the adult rat groups or between the young adult and adolescent rats. THC produced greater effects in females than males: Sex × THC Dose, $F(5, 440) = 5.24$, $p < .001$, and there were no Sex × Age or Sex × Age × THC Dose interactions. Sex differences in maximal paw pressure effect are shown in Table 1.

THC Effects on Locomotor Activity

Figure 4 shows that THC suppressed activity more in young adult than in adolescent and middle-aged rats: Age × THC Dose, $F(10, 440) = 5.03$, $p < .001$, with a somewhat greater age-related difference in females than males: Sex × Age, $F(2, 88) = 3.00$, $p = .055$. Given that THC suppressed locomotion more in females than in males: sex, $F(1, 88) = 9.01$, $p = .003$, further analyses were conducted within each sex. In females, THC suppressed locomotor activity significantly more in young adult than in adolescent and middle-aged adult rats: Age × THC Dose, $F(10, 215) = 4.63$, $p < .001$; Tukey post hoc: young adult vs. adolescent: $p = .025$; young adult vs. middle-aged adult: $p = .02$. In males, age group differences were smaller but still significant: Age × THC Dose, $F(10, 225) = 2.04$, $p = .03$, although post hoc tests revealed no significant differences between age groups at specific doses (all ps $> .05$).

Correlation analyses within each age and sex revealed a significant, moderate association between tail withdrawal and locomotor scores in all female groups (rs $= -.31$ to -0.51, $p = .002$ to $< .001$) and in adolescent and young adult male groups ($r = -.29$, $p = .004$; $r = -.25$, $p = .016$, respectively). A weaker but significant association between paw pressure and locomotor scores was observed in all female groups (rs $= -.27$ to $-.38$, $p = .007$ to $< .001$) but not in any of the male groups.

Serum THC and Metabolites

To determine whether the significantly lesser antinociceptive effects of THC in adolescent rats could be due to lower circulating THC and/or active metabolite levels in adolescent compared to adult rats, we determined serum levels of THC, its major active metabolite 11-OH-THC, its minor active metabolite cannabinol (CBN), and its major inactive metabolite THC-COOH from blood samples taken immediately after the last behavioral test, from a subset of adolescent and young adult rats ($n = 6$/sex/age). Figure 5 (top left panel) shows that serum THC levels actually tended to

Table 1
Maximal THC Effect (in %MPE) on Tail Withdrawal (TW) and Paw Pressure (PP) Tests in Rats by Age and Sex

	Adolescent (PND 35–40)		Young adult (PND 60–70)		Middle-aged adult (PND 291–325)	
Variable	Male	Female	Male	Female	Male	Female
TW	7.8 ± 2.1	26.2 ± 6.5[a]	40.9 ± 9.2	76.7 ± 7.0[a]	60.6 ± 9.5	89.6 ± 4.9[a]
PP	13.0 ± 10.2	42.2 ± 8.5[a]	38.9 ± 10.3	55.2 ± 9.2	40.3 ± 11.2	78.3 ± 9.9[a]

Note. THC = Δ^9-tetrahydrocannabinol; %MPE = percent maximum possible effect; PND = postnatal day.
[a] Maximal effect is significantly greater in females than in males at same age ($p < .05$).

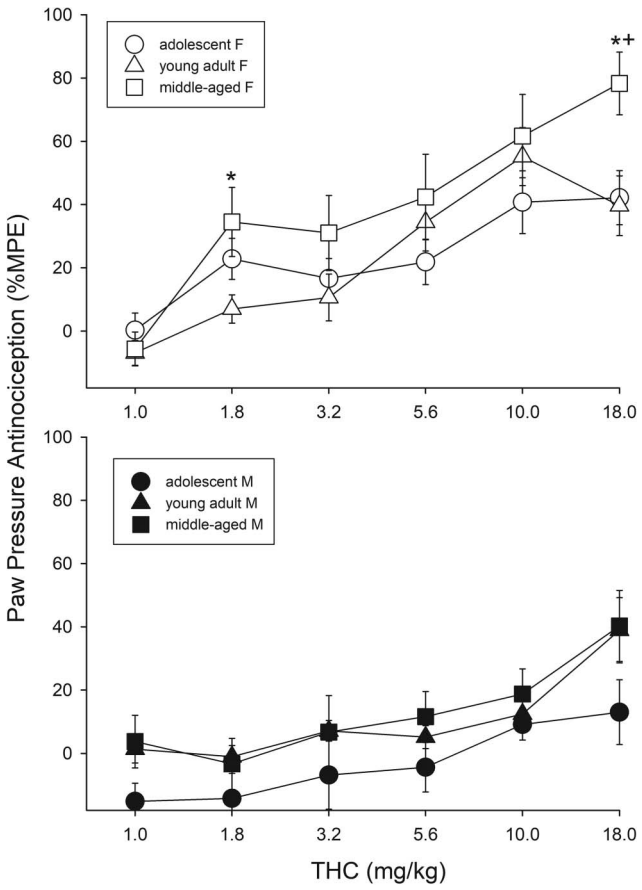

Figure 3. Age-dependent Δ^9-tetrahydrocannabinol (THC)-induced antinociception on the paw pressure test in female rats (top panel; $n = 14–16$ per age group) and male rats (bottom panel: $n = 16$ per age group). Asterisks indicate significantly different from the young adult group at the same dose; plus signs indicate significantly different from the adolescent group at the same dose (Tukey post hoc test, $p < .05$). Error bars indicate standard error of the means. F = female; M = male; %MPE = percent maximum possible effect.

be higher in adolescent than in young adult rats, although this age difference was not significant: Age, $F(1, 20) = 4.29$, $p = .052$; Age × Sex, $F(1, 20) = 2.13$, $p = .16$. Levels of the major active metabolite 11-OH-THC did not differ between adolescent and young adult rats, although they were significantly higher in females than in males (see Figure 5, top right panel): sex, $F(1, 20) = 15.18$, $p = .001$. The minor active metabolite CBN also did not differ between adolescent and young adult rats of either sex (see Figure 5, bottom left panel). Finally, serum levels of the major inactive metabolite THC-COOH did not differ between adolescent and young adult rats, although levels were higher in females than in males (see Figure 5, bottom right panel): sex, $F(1, 20) = 8.24$, $p = .009$.

Discussion

The main findings in the present study are (a) on acute thermal and pressure pain tests, THC produced significantly less antinociception in adolescent than in adult rats; (b) on the thermal pain test, THC also produced greater antinociception in middle-aged adult than in young adult rats; (c) age-related differences in THC-induced antinociception were not paralleled by age-related differences in THC-induced sedation; (d) serum levels of THC and metabolites did not differ significantly between adolescent and young adult rats; and (e) THC produced greater antinociception and locomotor suppression in female compared to male rats, which may be related to females' greater production of 11-OH-THC, the major active metabolite of THC.

We are aware of two previous studies in which antinociceptive effects of cannabinoids were compared between individuals of different ages. First, a single dose of anandamide or THC (20 mg/kg ip) increased latency to respond on a hotplate test in adult mice (6–8 weeks old, mixed sexes) but had no effect in preweanling mice (PND 6–23, mixed sexes; Fride & Mechoulam, 1996). Second, Wiley and colleagues (2007) compared THC's effects between early adolescent (PND 29) and young adult (PND 68) rats using a tail flick test. THC (1–180 mg/kg ip) lengthened tail-flick latency in all groups but was significantly more potent in adult

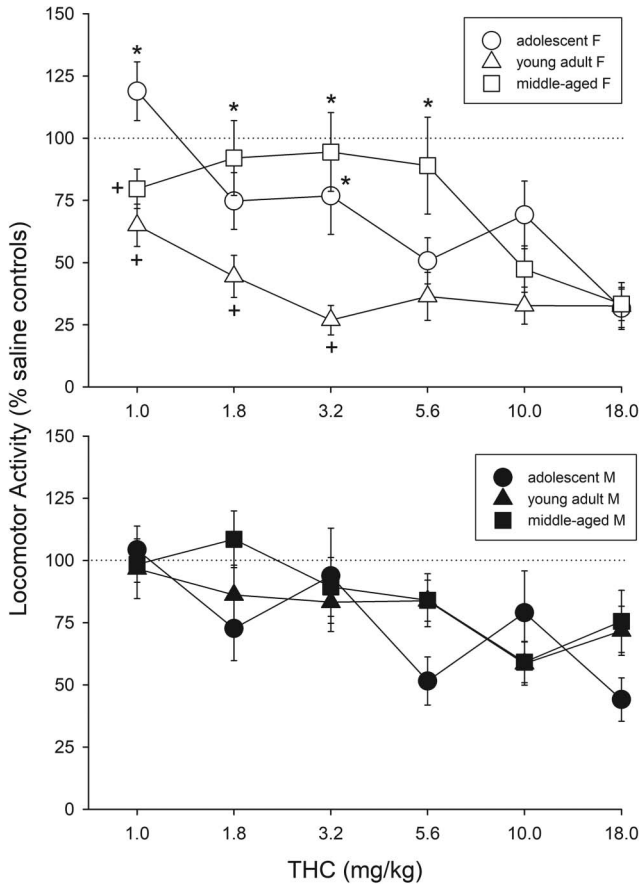

Figure 4. Age-dependent Δ^9-tetrahydrocannabinol (THC)-induced locomotor suppression in female rats (top panel; $n = 14–16$ per age group) and male rats (bottom panel: $n = 16$ per age group). Asterisks indicate significantly different from the young adult group at the same dose; plus signs indicate significantly different from the adolescent group at the same dose (Tukey post hoc test, $p < .05$). Error bars indicate standard error of the means. F = female; M = male.

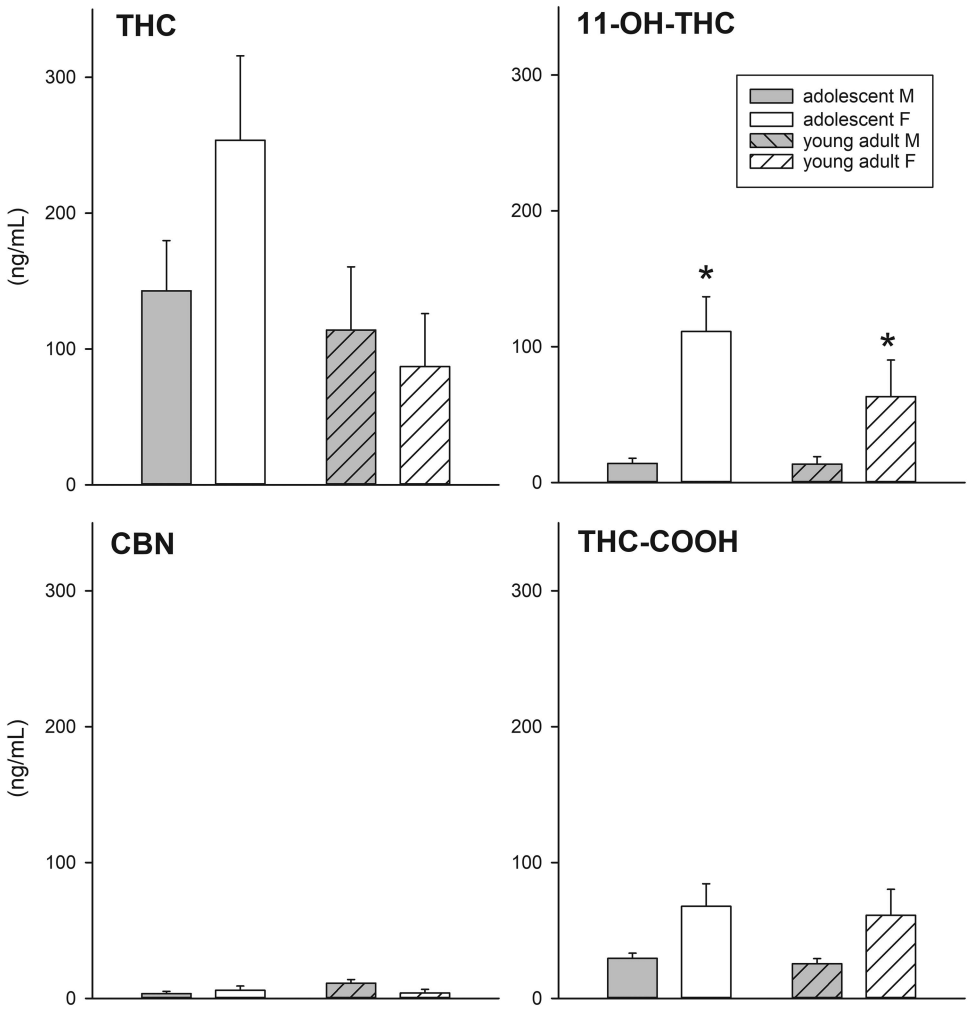

Figure 5. Δ^9-tetrahydrocannabinol (THC) and metabolite concentrations in blood samples taken immediately after completion of behavioral testing (approximately 20 min after the last injection; a total cumulative THC dose of 18 mg/kg ip). Asterisks indicate significant sex difference ($p < .05$). Error bars indicate the mean ± 1 *SEM* of six rats. M = male; F = female; CBN = cannabinol; THC-COOH = 11-nor-9-carboxy-THC.

than in adolescent female rats, with no age-related difference in males (Wiley et al., 2007). Thus, the present results agree to some extent with those of previous studies suggesting that rodents become more sensitive to cannabinoid antinociception with age, at least from the pre- and periadolescent period to young adulthood. One possible explanation for the discrepant result in male rats between our study and that of Wiley and colleagues is that we bred our rats in-house, whereas their rats were shipped from a breeder. Wiley and Evans (2009) reported that shipping weanlings from a commercial breeder altered antinociceptive sensitivity to THC when rats were tested as adolescents, in a sex-dependent manner: Compared to same-sex rats that had been shipped, males bred in-house tended to be less sensitive to THC, whereas females bred in-house tended to be more sensitive to THC. Thus, the fact that rats in our study were bred in-house may explain the lesser THC sensitivity we observed in adolescent compared to adult male rats.

To our knowledge, there are no published studies comparing cannabinoid antinociception in young versus older adult rodents or humans, so our finding of greater THC-induced antinociception in middle-aged compared to young adult rats (tail withdrawal test) appears to be novel. Past-year cannabis use among middle-aged U.S. adults (50–64 years old) increased 57.8% from 2006 to 2013 (Han et al., 2017); given that one of the more commonly reported medical uses of cannabis is pain relief (Sexton et al., 2016; Walsh et al., 2013), it is likely that some of the increase in cannabis use among middle-aged adults is for pain relief. The present results suggest that THC may be a particularly effective analgesic in middle-aged adults, especially women. Although greater cannabinoid analgesia in middle-aged adults could be fortuitous given their higher rates of chronic pain compared to young adults (Gibson & Lussier, 2012), therapeutic effects must be weighed against deleterious side effects. The present study demonstrates that sedation, a common side effect produced by THC, does not tend to be greater in middle-aged adult compared to young adult rats. It will be important to conduct a more comprehensive examination of side effects of cannabis use in middle-aged to older adult humans

(Kaskie, Ayyagari, Milavetz, Shane, & Arora, 2017), as well as to determine whether the greater antinociceptive efficacy of THC observed in the present study extends to models of chronic pain.

Whereas THC's antinociceptive effects tended to be greater in middle-aged adult rats than in young adult rats and greater in young adult rats than in adolescent rats of both sexes, age-related differences in THC's locomotor-suppressant effect were sex-specific, with sedation in young adult females being greater than in middle-aged and adolescent females. These results suggest that (a) longer latencies to respond on nociceptive tests in adult compared to adolescent rats were not simply due to greater drug-induced sedation in adult rats and (b) age-related differences in THC effect are specific to the behavioral end point examined and are thus likely due to a pharmacodynamic rather than pharmacokinetic mechanism. In contrast, the significant correlation between antinociceptive responses and locomotor activity in all female groups for both nociceptive tests (but in only two of six cases in the male groups) indicates that sex differences in THC's sedative effects may contribute to sex differences in THC's effects on the nociceptive tests. Regarding previous studies of age-related differences in cannabinoid-induced sedation, Fride and Mechoulam (1996) reported no effect of 20 mg/kg anandamide or THC in preweanling (mixed-sex) mice, whereas this dose significantly decreased open field activity in adult (mixed-sex) mice. Similarly, THC (.5–2.5 mg/kg) decreased activity in adult (PND 64–66) but not early adolescent (PND 28) male rats (Schramm-Sapyta et al., 2007). Other studies have found that lower doses of THC (.03–1 mg/kg) increased locomotor activity more in early adolescent (PND 27) than in young adult (PND 65–70) rats (Wiley & Evans, 2009; Wiley, Evans, Grainger, & Nicholson, 2011), whereas higher THC doses decreased activity more in adolescent than in young adult rats (Wiley et al., 2007). The lowest dose we tested, 1.0 mg/kg, did tend to increase activity in adolescent female rats but not in other female or male groups; however, only adolescent female rats in our study were significantly less sensitive than adult female rats were to THC-induced sedation (male adolescent vs. adult rats did not differ significantly in locomotor response to THC). Again, sex-specific discrepancies among results of rat studies may relate to shipping status of the rats (Wiley & Evans, 2009).

The serum cannabinoid analysis indicates that greater THC-induced antinociception in young adult compared to adolescent rats was not due to higher serum levels of THC or its major active metabolites in young adult compared to adolescent rats. In fact, adolescent rats—particularly females—tended to have higher serum THC levels than adult rats did. There were also no age-related differences in serum levels of the major active metabolite 11-OH-THC, the minor active metabolite CBN, or the major inactive metabolite THC-COOH, suggesting that lesser antinociception in adolescents was not due to differential metabolism of THC (i.e., to its inactive metabolite rather than active metabolites). Serum 11-OH-THC levels were higher in females than males of both ages, a sex difference that has been reported previously (Britch, Wiley, Yu, Clowers, & Craft, 2017; Narimatsu, Watanabe, Yamamoto, & Yoshimura, 1991; Wiley & Burston, 2014) and which contributes to greater antinociceptive effects of THC in young adult female compared to male rats (Tseng, Harding, & Craft, 2004). Thus, greater THC-induced antinociception in female than male adolescent rats may be due to females' greater production of 11-OH-THC at this age.

Numerous studies have demonstrated age-related changes in the endocannabinoid system, particularly from early neonatal ages to adolescence (for a review, see Lee et al., 2016), suggesting that pharmacodynamic mechanisms could underlie age-related differences in THC-induced antinociception. For example, brain CB1R density has been shown to increase from early neonatal ages to adolescence and young adulthood in rats, in some brain areas (Belue et al., 1995; de Fonseca et al., 1993; McLaughlin, Martin, Compton, & Abood, 1994). However, we could find no data comparing adolescent versus adult rat CB1R in brain areas that specifically mediate cannabinoid antinociception, such as the amygdala, PAG, and RVM (Lichtman et al., 1996; Martin et al., 1999; Meng et al., 1998). A recent study examining very young rats (PND 10, 21, and 40) reported that CB1R immunoreactivity decreased in ventral PAG while increasing in RVM across this age range; however, CB1R immunoreactivity was significantly lower in midbrain samples taken from human adults compared to human infants (Kwok et al., 2017). Infant versus adult ages were not specified in the latter study, so it is possible that adults were aged (e.g., >65 years old). Some rat studies have demonstrated a decline in brain CB1R from young adults to older (>1–2 years old) adults (e.g., in the nucleus accumbens: Amancio-Belmont, Romano-López, Ruiz-Contreras, Méndez-Díaz, & Prospéro-García, 2017; in the basal ganglia: Romero et al., 1998; in the cerebellum and hypothalamus: Berrendero et al., 1998), but we could find no studies that specifically compared CB1R in pain-related brain areas between young adults and middle-aged adults. Furthermore, because systemically administered cannabinoids produce antinociception by acting at multiple sites from the periphery to spinal cord to brain (Starowicz & Finn, 2017), considerably more research will be needed to pinpoint the full range of age-related changes in the endocannabinoid system that may underlie age-related differences in antinociceptive sensitivity to cannabinoids.

Age-related differences in other behavioral effects of cannabinoids have been observed in some but not all studies. For example, acute administration of WIN55,212–2 decreased novelty preference in adult (PND 70) male rats but not in adolescent (PND 35) male rats (Fox, Sterling, & Van Bockstaele, 2009). In contrast, whereas there was no effect of THC in adolescent or adult male mice in a novel object recognition task, age-dependent THC effects were observed on an elevated plus maze and in an open field test—although age differences were also mouse strain−dependent (Kasten, Zhang, & Boehm, 2017). Considerably more comparisons across broader age ranges will be needed to determine whether age-related effects of cannabinoids are limited to primarily early stages of life (birth to young adulthood) and whether they relate to brain site-specific developmental trajectories in the endocannabinoid system.

Two caveats bear mention in interpreting the present results. First, assuming that baseline response latency reflects how painful a noxious stimulus is, the shorter baseline tail withdrawal latencies in adolescent compared to adult rats (see Figure 1) suggest that the 50 °C water was more painful for adolescent than for adult rats (perhaps because a greater proportion of adolescents' tails was submerged in the water, compared to adults' tails: 80% of tail length compared to 40%–50%, respectively). A partial agonist like THC would be expected to be less effective against more intense pain than against less intense pain, so greater pain in adolescent

compared to adult rats may explain why THC was less effective in adolescent than in adult rats on this test. That is, the particularly large differences in THC effect between adolescent and adult rats on the tail withdrawal test may be due to greater sensitivity of adolescents than adults to that noxious stimulus. It is unlikely, however, that this is the sole reason for lesser antinociception in adolescent compared to adult rats, because on the paw pressure test, adolescent male rats actually had longer baseline latencies than adult male rats did yet still showed less THC-induced antinociception. Alternatively, it is possible that the larger age-related differences on the tail withdrawal test compared to the paw pressure test are due to greater age-related differences in spinal as opposed to supraspinal endocannabinoid mechanisms, given that the tail withdrawal reflex is primarily spinally mediated whereas paw withdrawal responses require supraspinal integration (Morgan, Sohn, & Liebeskind, 1989).

A second caveat relates to the age-dependent locomotor suppression produced by THC in females. THC-induced locomotor suppression was greater in young adult female than in adolescent and middle-aged female rats, but young adult females also showed significantly different locomotor activity than other groups under vehicle control conditions did (see Figure 1). We did not document estrous stage, so we cannot rule out its possible impact on activity in the young adult female group. It is possible that all or most females in the young adult control group happened to be in estrus, the stage during which young adult females are most active (Scimonelli, Marucco, & Celis, 1999). Because THC effects on activity were characterized relative to activity in vehicle-treated controls, a particularly active control group could exaggerate group differences in THC-induced locomotor suppression. In contrast, the potential confound of estrous stage on activity would not likely be an issue for the adolescent and middle-aged female groups, which would not be expected to be cycling regularly (Goldman, Murr, & Cooper, 2007; Scimonelli et al., 1999).

Conclusion

There have been no controlled studies of cannabinoid analgesia in human adolescents or children: A recent review lists a single case report of nabilone treatment for neuropathic pain in two adolescents (Wong & Wilens, 2017). Additionally, a review of medical cannabinoid studies that included older adults yielded insufficient data to determine analgesic efficacy in older adults (van den Elsen et al., 2014). The present study demonstrates age-related differences in THC-induced antinociception in both female and male rats that are not readily explained by age differences in THC's sedative effect or serum levels of THC or its major metabolites. Taken together with previous studies documenting a developmental trajectory in the mammalian brain endocannabinoid system, the present results suggest that the pain-relieving effects of THC may be limited in adolescent compared to adult pain patients and may also differ slightly between young and middle-aged adults. Given the broadening age range of cannabis users, the common use of cannabis to alleviate pain, and the increase in chronic pain with age, it will be important to determine whether the present findings extend to models of chronic pain and whether the therapeutic window for cannabinoid-based analgesics varies among individuals of different ages.

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