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Original Reports

Tetrahydrocannabinol (THC) Exacerbates Inflammatory Bowel Disease in Adolescent and Adult Female Rats

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Abstract: **Inflammatory Bowel Disease (IBD) is a life-long disorder that often begins between the ages of 15 and 30. Anecdotal reports suggest cannabinoids may be an effective treatment. This study sought to determine whether home cage wheel running is an effective method to assess IBD, and whether Tetrahydrocannabinol (THC), the primary psychoactive compound in cannabis, can restore wheel running depressed by IBD. Adolescent and adult female Sprague-Dawley rats were individually housed in a cage with a running wheel. Rats were injected with trinitrobenzene sulphonic acid (TNBS) into the rectum to induce IBD-like symptoms. One day later, both vehicle and TNBS treated rats were injected with a low dose of THC (0.32 mg/kg, s.c.) or vehicle. Administration of TNBS depressed wheel running in adolescent and adult rats. No antinociceptive effect of THC was evident when administered 1 day after TNBS. In fact, administration of THC prolonged TNBS-induced depression of wheel running for over 5 days in adolescent and adult rats. These results show that home cage wheel running is depressed by TNBS-induced IBD, making it a useful tool to evaluate the behavioral consequences of IBD, and that administration of THC, instead of producing antinociception, exacerbates TNBS-induced IBD.**

Perspective: ***This article advances research on inflammatory bowel disease in two important ways: 1) Home cage wheel running is a new and sensitive tool to assess the behavioral consequences of IBD in adolescent and adult rats; and 2) Administration of the cannabinoid THC exacerbates the negative behavioral effects of IBD.***

Key words: *Cannabis, visceral pain, wheel running, pain-depressed behavior, antinociception.*

Introduction

Inflammatory Bowel Disease (IBD) is a debilitating condition characterized by diarrhea, pain, and weight loss.[7] It is a lifelong condition that often begins between the ages of 15 and 30. The primary focus of treatment is to reduce inflammation either by administering corticosteroids or immunosuppressants. Treatments targeting pain are limited because standard pain treatments such as non-steroidal anti-inflammatory drugs (NSAIDs) and opioids have negative effects on the gastrointestinal tract. The use of animal models of IBD, such as intracolonic administration of 2,4,6-trinitrobenzenesulfonic acid (TNBS)[2,22] have been developed to better understand the underlying mechanisms and test new treatments.

TNBS-induced IBD produces clinically relevant changes in the bowel.[2,3] The degree to which this model produces spontaneous pain is not clear. Spontaneous pain is a defining symptom of IBD in humans, but difficult to assess in animals. The abdominal withdrawal reflex caused by colon distention has been used to assess visceral pain in animals,[26,49] but this method does not reveal the presence of spontaneous pain and has limited clinical relevance. In contrast, a wide range of pain conditions have been shown to depress wheel running in rats and mice.[17] One of the objectives of this study is to determine whether TNBS-induced IBD depresses home cage wheel running. Given that IBD often develops during adolescence, the effect of TNBS-induced IBD on home cage wheel running will be assessed in adolescent and adult rats.

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Wheel running studies are particularly useful in drug development because the treatment goal, restoration of wheel running, is consistent with the clinical goal of restoring function.[41] The presence of endocannabinoids throughout the gastrointestinal tract along with the anti-inflammatory actions of cannabinoids suggest they may be an effective treatment for IBD.[11,23] Animal studies show that administration of cannabinoids reduce inflammation in the colon[21] and pain from colonic distension.[18,34] The present study will assess the clinical relevance of these findings by determining whether THC, the primary psychoactive ingredient in cannabis, can restore wheel running depressed by TNBS-induced IBD in rats.

Materials and Methods

Subjects

Experiments were conducted on 31 adolescent (40 – 44 days old at the start of the experiment) and 28 adult (60 – 70 days old) female Sprague-Dawley rats (Envigo, Livermore, CA). Female rats were selected because the incidence of IBD is comparable in men and women in the United States, but related health issues makes IBD especially challenging to treat in women.[32] Rats were housed 2 -3 to a cage prior to the experiment and individually when the experiment began. Animals were housed in an isolated test room for the duration of the experiment. The lights were on a 12:12 hour cycle with the dark phase beginning at 2:00 PM. No one entered the room except during the hour prior to the beginning of the dark phase. Food and water were provided *ad libitum*. This research was conducted in accordance with NIH guidelines for animal care. The experiment was approved by the Institutional Animal Care and Use Committee at Washington State University.

Inflammatory Bowel Disease

IBD was induced by administration of trinitrobenzene sulphonic acid (TNBS) into the colon while the rat was anesthetized with isoflurane.[2] Adolescent rats were injected with 0.5 ml and adult rats with 0.6 ml of TNBS at a concentration of 30 mg/ml in saline and EtOH at a ratio of 1:4:5. Control rats were injected with the same volume of saline into the colon. The injections were made by inserting 8 cm of PE60 tubing into the rat's colon. Each rat was held upside down by the tail for 30 s after the injection to prevent TNBS from leaking out, and then the rat was returned to its home cage. The injections were completed 20 - 40 minutes prior to the beginning of the dark phase.

Dependent Variables

Home-cage wheel running was used to assess voluntary activity. A 28 cm diameter Kaytee Run-Around Giant Exercise Wheel (Kaytee Products, Inc., Chilton, WI) was suspended from the top of the rat's home cage. PAS software (San Diego Instruments, San Diego, CA) was used to automatically count wheel revolutions each time a small aluminum plate attached to a spoke of the wheel disrupted a photobeam. Wheel revolutions were assessed in 5-minute bins for 23 hours a day. Data were summed and analyzed in 3 and 12 hour time blocks to reduce variability from the intermittent nature of running and resting and differences in running during the dark and light phases of the circadian cycle. Body weight was assessed daily during the hour prior to the beginning of the dark phase.

Experimental Design

Each rat was housed individually in a cage with a running wheel for 7 days prior to assessment of baseline activity. At the end of the 23 hr baseline assessment, rats were anesthetized with isoflurane and injected with TNBS or saline into the colon and returned to their home cage. The following day rats were injected with THC (0.32 mg/kg, s.c.) or vehicle. Injections were completed 10 to 15 minutes prior to the beginning of the dark phase of the circadian cycle. Home cage wheel running was assessed for 5 days following the THC/vehicle injection.

Statistical Analysis

Data are presented as mean ± SEM. Baseline differences in wheel running between adolescent and adult rats were assessed using t-tests and ANOVA depending on the number of groups compared. The effects of TNBS and THC in adolescent and adult rats were analyzed separately using a repeated measures 2 (TNBS vs Saline) x 2 (THC vs vehicle) x 6 (Day) ANOVA. These data were converted to a percent of baseline to account for high individual variability in wheel running. Changes in body weight from baseline underwent a similar analysis. Statistical significance was defined as a probability of less than .05.

Results

Baseline running during the dark phase of the circadian cycle was significantly higher for adult compared to adolescent rats ($F(1,49)= 9.513$, $P = .003$). Adult rats had a mean of 3406 ± 458 revolutions during the 23 hour baseline period with an average of 96% of this running occurring during the 12 hour dark phase. Mean wheel revolutions for adolescent rats was 2181 ± 379 with an average of 87% of the running occurring during the dark phase. The higher levels of running in adult compared to adolescent rats were evident throughout the dark phase of the circadian cycle (Fig 1). Subsequent data analysis and graphs focus on differences in activity during the dark phase because the lack of running during the light phase imposes a floor effect.

TNBS or vehicle was injected into the colon during the hour after baseline assessment. Feces and/or TNBS leaked from the colon of 8 of the 17 adolescent rats and 2 of the 16 adult rats following administration. The impact of a reduced TNBS dose from leaking was

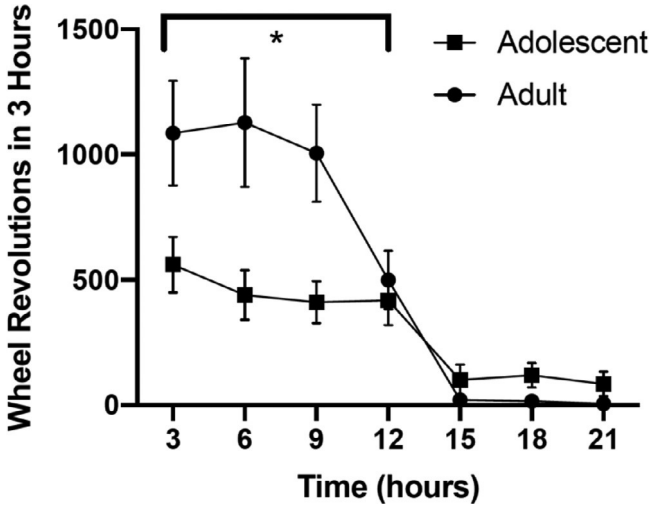

Figure 1. Adult rats have higher baseline levels of voluntary running than adolescent rats. Baseline wheel running was assessed for 23 hours prior to administration of TNBS. Mean wheel revolutions for adult (n = 20) and adolescent (n = 30) rats were summed in 3 hour blocks. Running is highest during the dark phase of the circadian cycle (3 – 12 hours). Running was much lower in both groups during the light phase (15 – 21 hrs). . * indicates a significant main effect.

evident both on wheel running and body weight. The eight adolescent rats in which TNBS leaked had 3049 wheel revolutions in the 23 hours following administration compared to 124 wheel revolutions in adolescent rats in which TNBS did not leak ($t(15) = 2.874$, $P = .01$). Moreover, adolescent rats with leaking TNBS lost 6.0 g in the day following administration compared to 16.8 g in rats that received the full TNBS dose ($t(15) = 3.302$, $P = .005$). Because of this difference subsequent data analysis and graphs only include rats that received the full TNBS dose. Although excluding these rats reduced the sample size to 4 and 5 adolescent rats in the TNBS-Vehicle and TNBS-THC groups, respectively, the magnitude of the effects precluded the need to test more rats.

The acute antinociceptive effect of THC administration on wheel running was assessed during the 3 hours following THC or vehicle administration. These data were converted to a percent of baseline running by dividing the number of wheel revolutions following THC or vehicle administration by the number of revolutions each rat recorded during the first 3 hours on the baseline day (ie, the day before TNBS administration). The mean 3 hr baseline data was relatively consistent across the four adolescent groups (Means = 444 – 551 revolutions) despite a lot of individual variability (4 – 1943 revolutions in 3 hrs). Administration of THC caused a significant reduction in wheel running in TNBS treated rats as indicated by a significant TNBS by THC interaction ($F(1,18) = 4.472$, $P = .049$) (Fig 2, left). Administration of vehicle caused 2 adolescent rats previously treated with TNBS to become active, but this activation was confined to the 3 hour test period as indicated by a decrease in wheel running when assessed over the full 12 hr dark phase (see Fig 3).

Wheel running during the 3 hour mean baseline period for the four adult groups ranged from means of 491 to 941 revolutions with individual levels of running ranging from 164 – 2156 revolutions in 3 hours. Adult rats treated with TNBS were significantly less active than saline treated rats during the 3 hour test period as indicated by a significant main effect of TNBS ($F(1,22) = 12.222$. $P = .002$) (Fig 2, right). Administration of THC in adult rats had no effect on wheel running whether rats had IBD or not as indicated by the lack of a significant main effect of THC ($F(1,22) = .053$, $P = .82$) or TNBS by THC interaction ($F(1,22) = .017$, $P = .898$).

A day to day analysis reveals a depression of wheel running in rats treated with TNBS that is prolonged in rats treated with THC. A pronounced depression of wheel running occurred in the adolescent ($t(21) = 7.229$, $P < .001$) and adult ($t(24) = 4.890$, $P < .001$) rats during the 12 hour dark phase following treatment with TNBS compared to vehicle (Day 1 in Fig. 3A & B). Half of the TNBS treated rats and half of the saline treated rats were injected with THC or vehicle on Day 2 resulting in four groups. A gradual and progressive recovery of wheel running occurred in adolescent rats treated with saline after TNBS until running was comparable to non-TNBS treated rats by Day 4 or 5 (Fig 3A). This recovery did not occur in rats treated with THC after TNBS. THC administration exacerbated TNBS-induced depression of wheel running as demonstrated by a significant TNBS/THC interaction ($F(1,19) = 8.470$, $P = .009$).

Administration of TNBS into the colon of adult rats caused a prolonged depression of wheel running during the dark phase of the circadian cycle as indicated by a significant main effect of TNBS ($F(1,22) = 19.690$, $P < .001$). Wheel running recovered from a low of 13% of baseline following administration of TNBS to 47% of baseline by the sixth day (Fig 3B). A similar recovery was not evident in rats treated with THC after TNBS. Running levels in adult rats injected with THC on Day 2 remained around 20% of baseline levels on most of the 6 days of testing (Fig 3B). Administration of THC in rats without IBD had no effect on wheel running as demonstrated by the lack of a significant main effect of THC ($F(1,22) = .183$. $P = .673$).

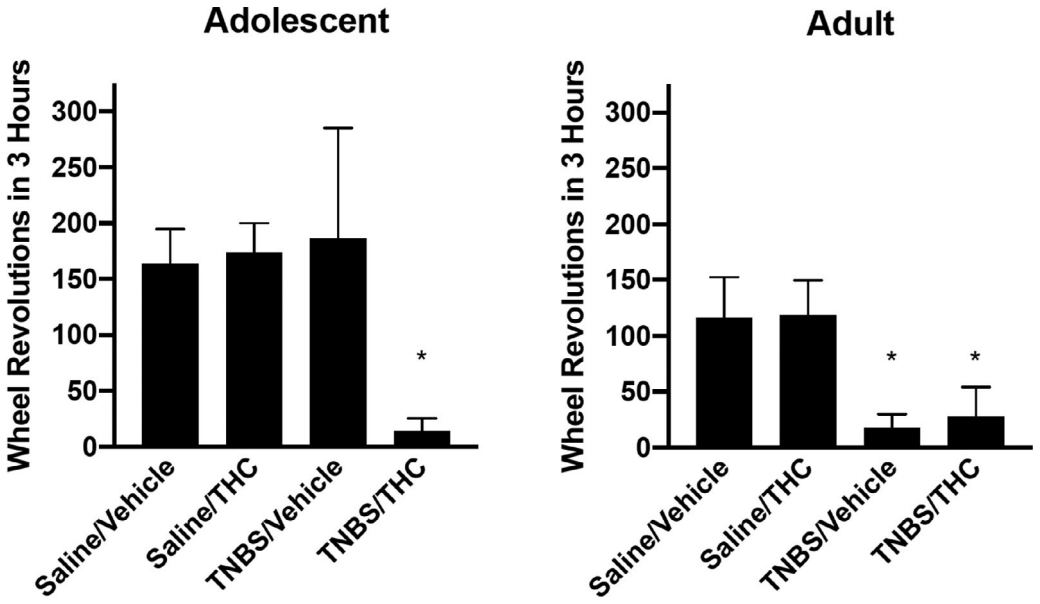

Figure 2. Administration of THC does not restore wheel running in rats with IBD. Data show the number of wheel revolutions as a percentage of baseline in the 3 hours following administration of THC or vehicle. Adolescent rats (left): Administration of THC reduced wheel running only in rats previously treated with TNBS (*Significant TNBS x THC interaction). Adult rats (right): Administration of TNBS reduced wheel running whether rats were treated with THC or not (*Significant main effect of TNBS). Data were collected during the first 3 hours of the dark phase on the second day after TNBS or saline administration into the colon. (N = 4 – 7 rats/condition).

Changes in body weight mimic the changes in wheel running. Administration of TNBS caused a significant decrease in body weight in both adolescent ($t(21) = 7.776$, $P < .0001$) and adult ($t(24) = 8.428$, $P < .001$) rats (Fig. 3C & D). Adolescent rats treated with TNBS and THC lost significantly more weight than rats treated with TNBS alone as indicated by a significant interaction ($F(1,19) = 39.194$, $P < .001$; Fig 3C). As with wheel running, TNBS induced weight loss in adult rats was comparable whether rats were treated with THC or vehicle as evident by the lack of a significant interaction between these factors ($F(1,22) = .015$, $P = .904$; Fig 3D). There was a significant main effect of TNBS on body weight across the 6 days of testing ($F(1,22) = 67.384$, $P < .001$).

Discussion

The present results suggest that home cage wheel running is an effective non-invasive method to assess the disruptive effects of IBD in rats. Although administration of TNBS depressed wheel running in both adolescent and adult rats, the magnitude and duration of this depression was greater in adult than adolescent rats. TNBS administration also caused a loss in body weight that closely matched the changes in wheel running. Contrary to expectations, not only did administration of THC lack antinociceptive activity, it prolonged TNBS-induced depression of wheel running and weight loss. These changes were particularly noticeable in adolescent rats.

TNBS-induced IBD is one of many pain conditions that has been shown to depress wheel running. Other pain conditions include inflammation, arthritis, migraine, corneal abrasion, surgery, neuropathy, and pancreatitis.[4,9,13,14,16,17,28,29,38,48] The magnitude and duration of TNBS-induced depression of wheel running in adult rats is comparable to the decrease that occurs following induction of hind paw inflammation.[14] Although pain is a major symptom of IBD and pain depresses wheel running, other symptoms of IBD such as fatigue or a general disruption of well-being may contribute to this decrease in activity.[7] In contrast to adults, adolescent rats recovered to control levels of wheel running 4 to 5 days after TNBS administration. The difference in IBD time course is probably caused by the lower dose and volume of TNBS injected into adolescent compared to adult rats (15 mg/0.5 mL vs 18 mg/0.6 mL). Additional studies in adolescent rats are needed to elucidate any differences in disease state compared to adult animals.

The time course for the depression in wheel running in adult rats is consistent with early stage changes to the colon caused by TNBS administration such as an increase in inflammatory mediators, oxidative damage, changes in gene expression, thickening of the colon wall, and hemorrhage.[2,22,25,37,43,45] Some of these changes are evident within 4 hrs of TNBS administration and most of these effects resolve within 7 to 12 days when the colon transitions to chronic inflammation and ulceration—changes that may persist for weeks.[5,22] Hypersensitivity to distension of the colon has been reported out to 56 days after inflammation of the mouse colon with zymosan.[12] Less clear is how TNBS depression of wheel running corresponds to these late occurring changes in colon inflammation because little is known about colon inflammation in adolescent rats and we only measured changes in wheel running during the early stages of colon inflammation in adult rats. Changes in wheel running closely mimic the decrease in body weight reported here and in other studies which show a 10 to 20%

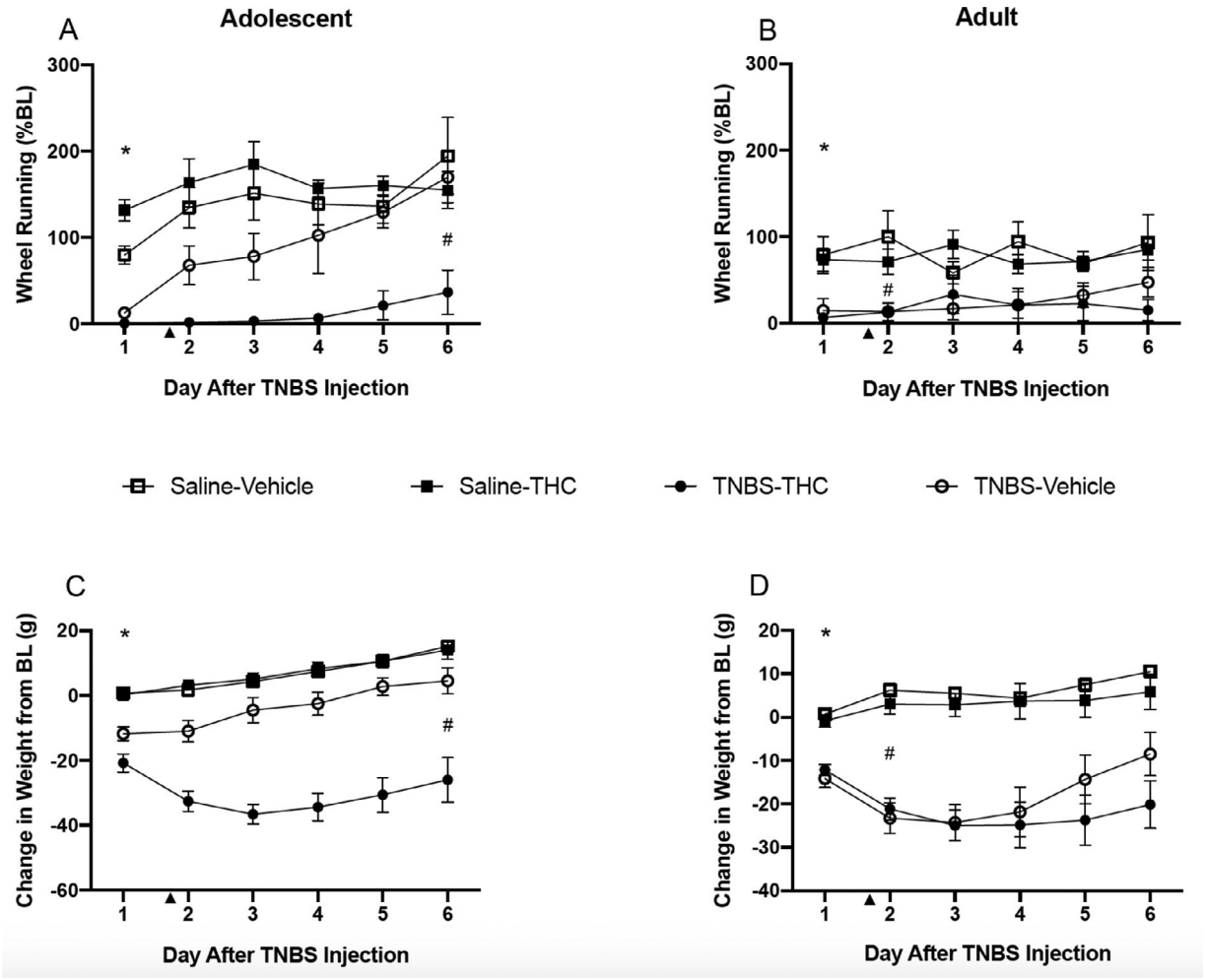

Figure 3. Administration of THC exacerbated the decrease in wheel running and weight loss caused by TNBS administration in adolescent (Left) and adult (right) rats. Daily wheel revolutions during the dark phase of the circadian cycle (percent of baseline) and change in body weight (change from baseline) across days following TNBS administration are displayed in the top and bottom graphs, respectively. Administration of TNBS caused a significant reduction in wheel running during the 12 hour dark phase immediately following TNBS administration (Day 1) in adolescent (**A**) and adult (**B**) rats (*t-test). Adolescent, but not adult rats recovered with time. Adolescent rats treated with THC (p) did not recover resulting in a significant TNBS by THC interaction (#). A significant main effect of TNBS was evident in adult rats (#) whether rats were treated with THC or not. Changes in body weight mimic the changes in wheel running. Administration of TNBS caused a large decrease in body weight in adolescent (**C**) and adult (**D**) rats (*t-test). Administration of THC (▲) enhanced weight loss in adolescent rats as indicated by a significant TNBS x THC interaction (#). Administration of THC had no effect on body weight in adults resulting in a significant main effect of TNBS (#). N = 4 – 7 rats/condition.

decrease in weight that occurs during the first week following TNBS administration.[22,35]

Whether pain or a more general malaise is the primary cause of depressed wheel running is not clear and may not matter. The decrease in wheel running caused by TNBS administration models the disruptive effect of IBD in humans. IBD greatly reduces quality of life in humans, including a reduction in voluntary activity.[7,27] The treatment goal—restoration of normal activity—is consistent in both situations. An increase in activity may facilitate recovery. Although few studies have examined the effect of exercise on IBD in humans, there appears to be a mild beneficial effect.[6,8] Exercise may have contributed to the rapid recovery in adolescent rats, although baseline levels of activity were lower in adolescent than adult rats and the voluntary nature of home cage wheel running limits the impact of exercise because rats with IBD run very little.

Current treatments for IBD focus on reducing inflammation by administration of corticosteroids or immunosuppressants. Although many IBD patients self-medicate with cannabis,[20,31,47] clinical trials have found limited efficacy and, in some cases, negative effects.[10,24,30] The negative effects of cannabis may be particularly problematic for IBD patients with Crohn's Disease.[39] Our data are consistent with these negative effects. Administration of THC exacerbated TNBS-induced depression of wheel running in both adolescent and adult rats. This depression of wheel running was most evident in adolescent rats because adult wheel running was already quite low (ie, there was a floor effect in the adults). The negative impact of THC during this early stage of TNBS-induced IBD is similar to the negative effect of twice daily injections of non-steroidal anti-inflammatory drugs during the week following TNBS.[44]

The negative effect of THC on TNBS-induced depression of wheel running is surprising both because a single injection depressed wheel running for 5 days and THC appears to produce antinociception in other animal studies. THC may have a prolonged negative effect as a result of inhibiting gastrointestinal transit or blocking the inflammatory processes engaged in healing. It is also possible that previous studies reporting inhibition of pain-evoked responses (eg, hot plate and von Frey tests) to cannabinoid administration are caused by motor or sedative effects.[42] Direct comparison of pain-evoked and pain-depressed tests revealed THC inhibition of visceral pain reflexes, but no restoration of pain-depressed behavior,[19] indicating that the ability of cannabis to suppress behavior is greater than the antinociceptive effect. We used a low dose of THC (0.32 mg/kg) to avoid motor and sedative effects. Although this dose of THC has no effect on wheel running in pain-free rats,[15] it exacerbated TNBS-induced depression of wheel running.

Although pain treatment is one of the main reasons people self-medicate with cannabis,[36,40,46] randomized controlled trials reveal that the analgesic effects are relatively minor and limited to specific pain conditions. The best analgesic effects are reported for neuropathic pain.[1,33] Taken together, the analgesic effects of cannabinoids appear to be much more limited than previously described. Moreover, the present study shows that THC can have deleterious effects in some pain conditions. Of course, THC is just one of many psychoactive compounds in cannabis so the negative effects of THC could be offset or reversed when combined with other cannabinoids. Timing is another factor that may influence the effects of THC. THC may exacerbate IBD during the early phases of inflammation as reported here, but alleviate pain in response to chronic inflammation.

In conclusion, this manuscript advances research on IBD in 2 important ways. This study shows that home cage wheel running is an accurate and clinically relevant method to assess IBD in animals. Second, administration of THC exacerbates IBD in adolescent and adult rats. The use of home cage wheel running will enhance research on IBD by providing a continuous measure of disease severity. This approach would be especially useful in the development of new treatments in which recovery of function is a shared goal with clinical treatment.

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