Δ^9-tetrahydrocannabinol Attenuates Oxycodone Self-Administration Under Extended Access Conditions

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Running Title: THC / Oxycodone interactions

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Abstract

Growing nonmedical use of prescription opioids is a significant global problem which motivates research on ways to reduce therapeutic use and combat addiction. Medical marijuana availability has been associated epidemiologically with reduced opioid harms and cannabinoids have been shown to modulate effects of heroin or morphine in animal models. This study was conducted to determine if Δ^9-tetrahydrocannabinol (THC) enhances the rewarding and/or antinociceptive effects of oxycodone.

Male Wistar rats were trained to intravenously self-administer (IVSA) oxycodone (0.15 mg/kg/infusion) during 1 h or 8 h sessions. After acquisition of oxycodone IVSA, rats were exposed to THC by vapor inhalation (0, 100 or 200 mg/mL in the vehicle; 1 h and 8 h groups) or injection (0, 5 or 10 mg/kg, i.p., 8 h group) prior to IVSA sessions. Oxycodone intake was significantly decreased in rats following vaporized or injected THC compared with vehicle treatment prior to the session. Additional groups of male and female Wistar rats were assessed for nociception of a 52 °C hot water bath following inhalation of vaporized THC (50 mg/mL), oxycodone (100 mg/mL), the THC/oxycodone combination or the PG vehicle. Tail withdrawal latency was increased more by the THC/oxycodone combination compared to either drug alone. Similar additive effects on tail withdrawal latency were produced by injection of THC (5.0 mg/kg, i.p.) and oxycodone (2.0 mg/kg, s.c.).

These data show additive effects of THC and oxycodone in rats and suggest the potential use of cannabinoids to enhance therapeutic efficacy and to reduce non-medical opioid abuse.

Introduction

Non-medical opioid abuse is a significant global problem, with an estimated 33 million users of opiates and prescription opioids worldwide (UNODC, 2016). Approximately 2 million people in the US have a prescription opioid related abuse disorder (CBHSQ, 2015), which may increase the likelihood of later nonprescription opioid use (Muhuri, 2013), and prescription opioid related overdose deaths have drastically increased over the last two decades (CDC, 2016). Despite the growing impact of prescription opioids on public health, relatively few pre-clinical studies have investigated the self-administration of oxycodone, one of the most commonly prescribed medications (OxyContin® or as part of Percocet®). Available studies confirm that oxycodone self-administration causes behavioral changes (Zhang *et al*, 2016) sometimes physical dependence and withdrawal (Enga *et al*, 2016) in mice and that male and female rats acquire oxycodone self-administration at similar rates (Mavrikaki *et al*, 2017). Thus traditional IVSA models can be used to evaluate approaches to reduce prescription opioid abuse.

Indirect evidence suggests that marijuana may attenuate some of the harms associated with opioid use. Epidemiological studies found reductions in opioid positive drivers in car crash fatalities in younger drivers 21-40 (Kim *et al*, 2016) over non-medical marijuana states and in-patient hospitalization rates for opioid dependence were 23% lower in medical marijuana states compared with non-medical marijuana states (Shi, 2017). Opioid overdose mortality is lower in states with medical marijuana legalization (Bachhuber *et al*, 2014) and an experimental study found that inhalation of cannabis (via Volcano® vaporizer) decreased pain in chronic pain patients that were being maintained on extended release oxycodone or morphine without changing the plasma concentration-time curves for either medication (Abrams *et al*, 2011). These findings suggest that psychoactive cannabinoids may interact with the effects of opioids, both to enhance therapeutic impact and to potentially reduce nonmedical use.

Currently there is only limited direct evidence for the interactive effects of cannabinoid and opioid receptor signaling; a few preclinical studies have investigated whether cannabinoid receptor activation via CB_1 agonists, including Δ^9-tetrahydrocannabinol (THC), can modify the effects of heroin or morphine but no studies have investigated the combined effects of THC with oxycodone. Daily treatment with THC by injection decreases responding for intravenous heroin in rhesus monkeys (Maguire and France, 2016)

and similar effects were observed after injection of full agonist CB_1 ligands (Maguire *et al*, 2013). In addition, THC enhances the antinociceptive effect of morphine in mice (Pugh *et al*, 1996), and the cannabinoid receptor full-agonists CP55,940 and WIN 55,212 enhance antinociceptive effects of morphine in rhesus monkeys (Li *et al*, 2008). Inhibitors of endocannabinoid catabolic enzymes may attenuate heroin and morphine-induced anti-nociception and dependence in mice (Ramesh *et al*, 2013; Wilkerson *et al*, 2017). This predicts that THC may likewise interact with the effects of prescription opioids such as oxycodone.

Because THC is typically administered via inhalation in humans, it is of further interest to determine if the inhalation route of administration produces interactions with oxycodone in animal models. A new method for delivery of drugs to rats using e-cigarette technology has been recently reported (Nguyen *et al*, 2016a; Nguyen *et al*, 2016b) and, pursuant to this study, inhaled THC produced antinociceptive effects commensurate with those produced by 10 mg/kg THC, i.p. (Javadi-Paydar *et al*, 2017; Nguyen *et al*, 2016b). This study was therefore designed to determine if THC inhalation reduces oxycodone intravenous self-administration (IVSA) and increases oxycodone-induced anti-nociception in a rat model. An initial study was performed in a group of rats that had been vaccinated with an anti-oxycodone vaccine (Oxy-TT) or the carrier protein control (TT) and trained to IVSA in one-hour limited access sessions. This vaccination model results in about a 50% reduction in brain levels of oxycodone after a given dose (Nguyen *et al*, 2017b) and increases the intravenous self-administration of oxycodone under easy access conditions (i.e., a fixed-ratio 1 response contingency), albeit less than required to compensate for reduced brain levels. This group difference permits the evaluation of the hypothesis that THC enhances the rewarding value of the oxycodone, as opposed to altering behavior in a non-specific manner. Secondarily it serves as a test of combined vaccine and small molecule therapy for drug abuse, as proposed in a recent review (Hwang and Janda, 2017). The follow-up study used an extended-access model in which animals were trained to self-administer oxycodone in 8 h sessions as a stronger test of effects of THC on a compulsive-like behavioral phenotype (Vendruscolo *et al*, 2011; Wade *et al*, 2015). This latter group was evaluated for effects of both inhaled and injected THC on oxycodone self-administration which were contrasted with the effects of pre-injection with oxycodone or the mu opioid

receptor antagonist naloxone. Finally, male and female rats were evaluated on a nociception assay to determine any interactive effects of THC and oxycodone.

Methods

Subjects: Male (N=50) and female (N=8) Wistar (Charles River, New York) rats were housed in humidity and temperature-controlled (23±1 °C) vivaria on 12:12 hour light:dark cycles. Animals entered the laboratory at 10-11 weeks of age. Animals had ad libitum access to food and water in their home cages and all experiments were performed in the rats' scotophase. All procedures were conducted under protocols approved by the Institutional Care and Use Committees of The Scripps Research Institute and in a manner consistent with the Guide for the Care and Use of Laboratory Animals (National Research Council (U.S.). Committee for the Update of the Guide for the Care and Use of Laboratory Animals. *et al*, 2011).

Drugs: Oxycodone HCl and naloxone HCl were obtained from Sigma-Aldrich (St. Louis, MO). The Δ^9-tetrahydrocannabinol was obtained from NIDA Drug Supply. THC was prepared in a 1:1:8 ratio of ethanol:cremulphor:saline and oxycodone, buprenorphine and naloxone were dissolved in saline (0.9%) for injection. Drugs were dissolved in propylene glycol (PG) for vapor inhalation experiments with the concentrations expressed as mg of drug per mL of PG. Drug injections were administered, and vapor inhalation sessions were initiated, 30 min prior to the start of self-administration sessions.

Hapten Synthesis and Vaccine Formulation. The oxycodone hapten (Oxy) was designed with an activated linker extending from the bridgehead nitrogen to directly react with the surface lysines of carrier protein tetanus toxoid (TT) or BSA. Oxycodone hapten was synthesized according to previously published methods from the Janda laboratory with slight modification in the reductive amination and amide bond formation steps (Kimishima *et al*, 2016; Nguyen *et al*, 2017b). Vaccines were formulated the day of immunization using 13:1:5 (v/v/v) mixture of Oxy-TT (1.0 mg/ml in PBS) or control TT (1.0 mg/ml in PBS), CpG ODN 1826 (5 mg/ml in PBS), and Alhydrogel® (alum, 10 mg/ml, InvivoGen) and

administered intraperitoneally. The Group 1 rats were administered the conjugate vaccine (Oxy-TT; N=12) or tetanus toxoid only (TT; N=10) on Weeks 0, 2, 4, and 8. The immunization protocol was adapted from a vaccination protocol previously reported (Nguyen *et al*, 2016c; Nguyen *et al*, 2017a). Within the TT group 8 rats completed acquisition with patent catheters and7 completed the THC inhalation study. Within the Oxy-TT group, 11 completed the entire study.

Self-administration procedure

Drug self-administration was conducted in operant boxes (Med Associates) located inside sound-attenuating chambers located in an experimental room (ambient temperature 22 ± 1 °C; illuminated by red light) outside of the housing vivarium. To begin a session, the catheter fittings on the animals' backs were connected to polyethylene tubing contained inside a protective spring suspended into the operant chamber from a liquid swivel attached to a balance arm. Each operant session started with the extension of two retractable levers into the chamber. Following each completion of the response requirement (response ratio), a white stimulus light (located above the reinforced lever) signaled delivery of the reinforcer and remained on during a 20-sec post-infusion timeout, during which responses were recorded but had no scheduled consequences. Drug infusions were delivered via syringe pump. The training dose (0.15 mg/kg/infusion; ~0.1 ml/infusion) was selected from prior self-administration studies (Wade *et al*, 2015). Group 1 was trained in 1 h sessions under a Progressive Ratio (PR) response contingency for the initial 7 sessions and Fixed Ratio 1 thereafter. In the PR paradigm, the required response ratio was increased after each reinforcer delivery within a session (Hodos, 1961; Segal and Mandell, 1974) as determined by the following equation (rounded to the nearest integer): Response Ratio=5e^(injection number**j*)–5 (Richardson and Roberts, 1996). The *j* value was set to 0.2. This group was initially trained with a PR contingency since a prior study had found that the Oxy-TT vaccination resulted in higher IVSA oxycodone intake under FR response contingency but a greater reduction in drug intake associated with the increased workload of a PR paradigm. Group 2 (N=12) was trained in 8 h sessions using a Fixed Ratio 1 response contingency. Rats were trained during weekdays (5 days per week).

Inhalation Apparatus and Procedure:

Sealed exposure chambers were modified from the 259mm X 234mm X 209mm Allentown, Inc (Allentown, NJ) rat cage to regulate airflow and the delivery of vaporized drug to rats, as has been previously described (Nguyen *et al*, 2016a; Nguyen *et al*, 2016b). An e-vape controller (Model SSV-1; La Jolla Alcohol Research, Inc, La Jolla, CA, USA) was triggered to deliver the scheduled series of puffs from Protank 3 Atomizer (Kanger Tech; Shenzhen Kanger Technology Co.,LTD; Fuyong Town, Shenzhen, China) e-cigarette cartridges for the 8 h self-administration experiment. Type 2 sealed exposure chambers (La Jolla Alcohol Research, Inc; La Jolla, CA, USA) and a second generation e-vape controller (Model SSV-2; La Jolla Alcohol Research, Inc, La Jolla, CA, USA) with Herakles Sub Ohm Tank e-cigarette cartridges (Sense; Shenzhen Sense Technology Co., LTD; Baoan Dist, Shenzhen, Guangdong, China) by MedPC IV software (Med Associates, St. Albans, VT USA) were used for tail withdrawal and the 1 h self-administration experiments. The chamber air was vacuum controlled by a chamber exhaust valve (i.e., a “pull” system) to flow room ambient air through an intake valve at ~1 L per minute. This also functioned to ensure that vapor entered the chamber on each device triggering event. The vapor stream was integrated with the ambient air stream once triggered. For self-administration studies, rats were exposed to 30 min of THC vapor inhalation (followed by a 5 min period for chamber clearance) immediately prior to the start of self-administration sessions.

Nociception Assay:

Tail withdrawal anti-nociception was assessed using a water bath (Bransonic® CPXH Ultrasonic Baths, Danbury, CT) maintained at 52 °C.. The latency to withdraw the tail was measured using a stopwatch and a cutoff of 15 seconds was used to avoid any possible tissue damage (Wakley and Craft, 2011; Wakley *et al*, 2014). Tail withdrawal was assessed starting 35 minutes after the initiation of inhalation or 30 minutes after injection. Nociception experiments following injected oxycodone (1,2 mg/kg, s.c.), THC (5,10 mg/kg, i.p.) or the combination were conducted in a group of adult female (N=8; 48 wks of age, 665.2, SD 76.2 g) and male (N=6; 48 wks of age, 310.5, SD 44.3 g) Wistar rats that were previously used in experiments of chronic vapor inhalation of THC (Nguyen *et al*, 2017c). Nociception experiments

following vapor inhalation were conducted in a separate group of male Wistar rats (N=10; 44 wks of age, 722.6, SD 84.2 g), that were previously used in pilot experiments with the nociception assay following vapor inhalation of heroin, oxycodone, methadone and THC to determine exposure conditions.

Data Analysis

Analysis of IVSA data was conducted with repeated-measures Analysis of Variance (rmANOVA) on the number of infusions earned during the acquisition interval and during drug pretreatment studies.

Within-subjects factors of Session and Drug Treatment condition were included. A between-groups factor of vaccine treatment was included for the 1 h IVSA experiment. Significant main effects were followed with post hoc analysis using Dunnett, Tukey (multi-level factors) or Sidak (two-level factors) tests for multiple comparisons. A Grubbs test eliminated two individuals from Group 1 which exhibited outlier IVSA during the vapor-inhalation test (Intakes 2.9 SD greater than the mean were observed for one individual on the Air and one on the THC condition).Tail withdrawal latencies were analyzed with repeated measures ANOVA including within-subjects factors of Drug Treatment Condition and Time post-injection/initiation of vapor. All analysis used Prism

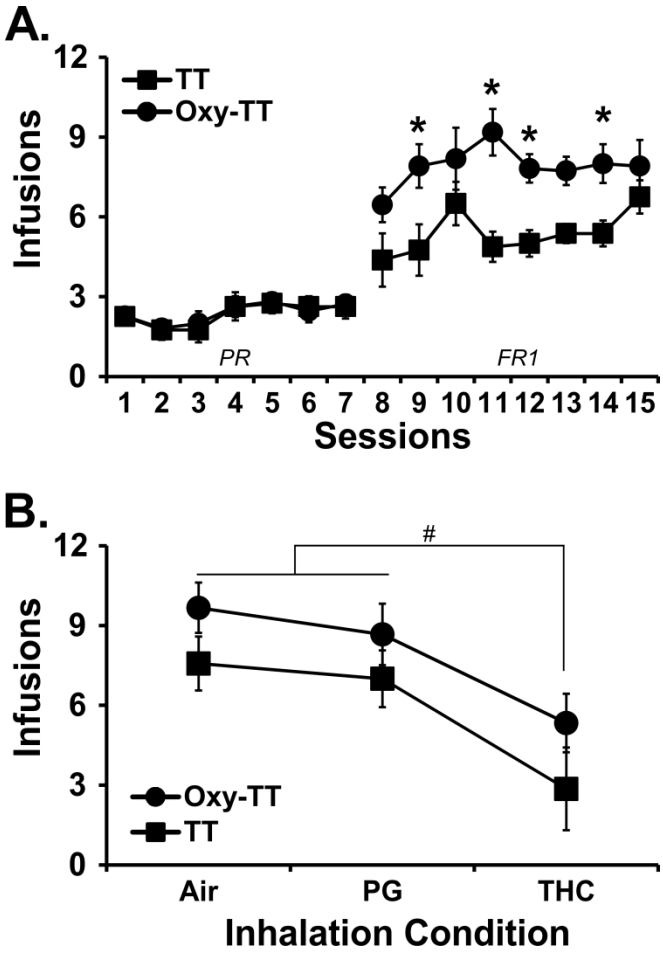

Figure 1. THC vapor inhalation reduces oxycodone self-administration. *A) Mean infusions obtained by groups of male rats vaccinated with the tetanus toxioid carrier protein (TT; N=8; ±SEM) or the anti-oxycodone conjugate vaccine (Oxy-TT; N=11; ±SEM) or trained to self-administer oxycodone (0.15 mg/kg/inf) within 1 h sessions under a Progressive Ratio (Sessions 1-7) or Fixed Ratio 1 (Sessions 8-15) response contingency. Significant differences within group from session 1 are indicated by *. B) Mean (TT, N=7; Oxy-TT, N=9; ±SEM) infusions following 30 minutes inhalation of Air, Propylene Glycol (PG) vehicle vapor or THC vapor. Significant differences from Air and PG vehicle condition are indicated by #.*

6 or 7 for Windows (v. 6.07 and 7.00; GraphPad Software, Inc, San Diego CA).

Results

THC vapor attenuates oxycodone self-administration under short access conditions

The TT (N=8) and Oxy-TT (N=11) vaccinated rats were trained to self-administer oxycodone across 15 session with group differences observed only under the FR1 response contingency (**Figure 1A**). Oxy-TT group self-administered more oxycodone during the FR1 phase of the acquisition, consistent with a sequestration of part of the dose in the bloodstream. The ANOVA confirmed significant effects of Session [F(14,238)=41.13; $p<0.0001$], of vaccine Group [F(1,17)=6.23; $p<0.05$] and of the interaction of Group with Session [F(14,238)=4.21; $p<0.0001$] on oxycodone intake. The post hoc test confirmed that the Oxy-TT group obtained more infusions for sessions 9, 11, 12, 14. In the critical study, it was found that THC vapor inhalation for 30 minutes significantly reduced (**Figure 1B**) the number of infusions of oxycodone obtained

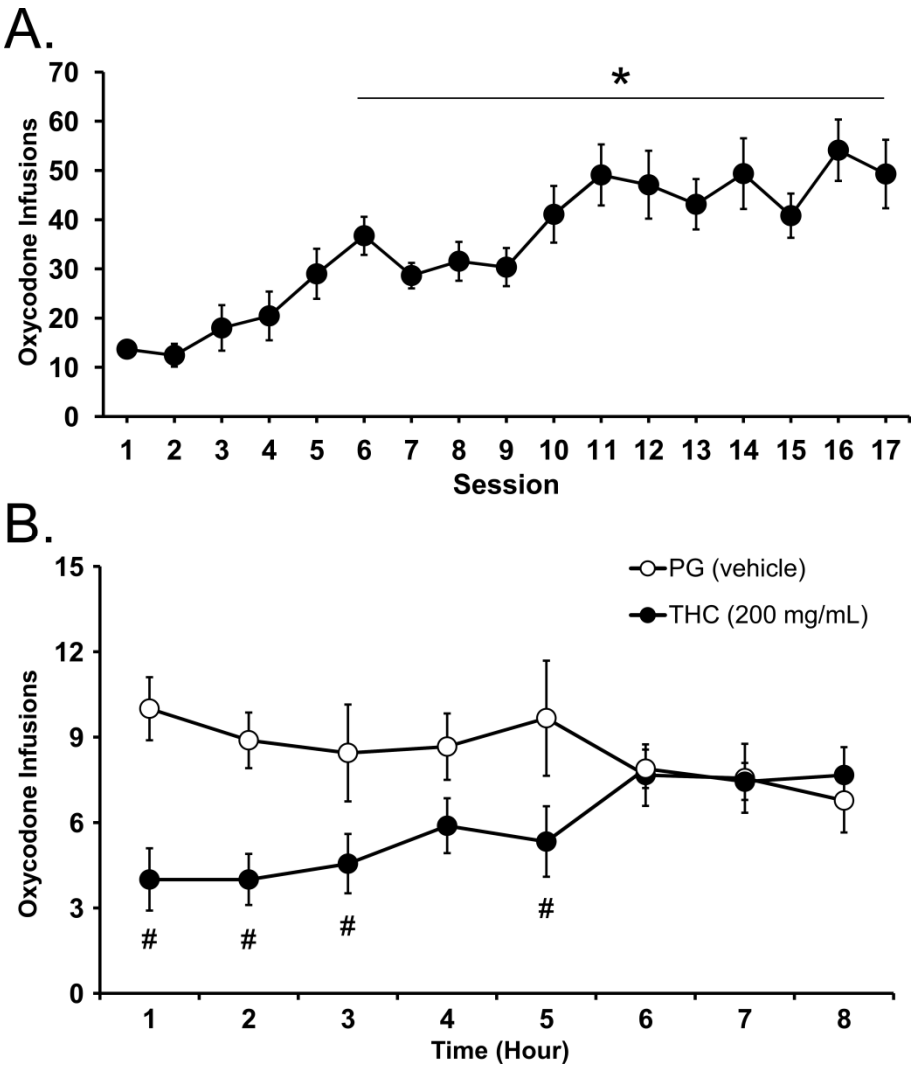

Figure 2. THC vapor inhalation reduces oxycodone self-administration. *A) Mean (N=11; ±SEM) infusions for male rats trained to self-administer oxycodone (0.15 mg/kg/inf) within 8 h extended access. B) Mean (N=11; ±SEM) infusions following THC vapor. Significant differences within group from session 1 are indicated by *. Significant differences from PG vehicle condition are indicated by #.*

(Significant main effect of Dose condition [F(2,28)=21.96; $p<0.0001$] but not of Group or the interaction of factors) relative to Air or PG inhalation (which did not differ from each other).

THC vapor attenuates oxycodone self-administration under extended access (8 h) conditions

Male rats (N=11) trained to self-administer oxycodone in 8 h sessions significantly escalated their intake during 17 sessions of acquisition training [F(3.255,32.55)=11.41; $p<0.0001$] as is shown in **Figure 2A**. The post hoc analysis confirmed significant increases in oxycodone intake relative to the first session across sessions 6-17. Inhalation of THC (200 mg/mL; 30 min) immediately prior to the self-administration session significantly reduced the mean (N=9; two rats were excluded due to mechanical failure on one of the test sessions) infusions of oxycodone compared to the effect of inhalation of the PG vehicle (**Figure 2B**). The ANOVA confirmed significant effect of vapor Treatment [F(1,8)=10.27; $p<0.05$] and of the interaction of factors [F(7,56)=5.587; $p<0.001$]. Post hoc analysis confirmed that oxycodone IVSA was reduced in hours 1-3 and 5 relative to the PG inhalation condition. There was no

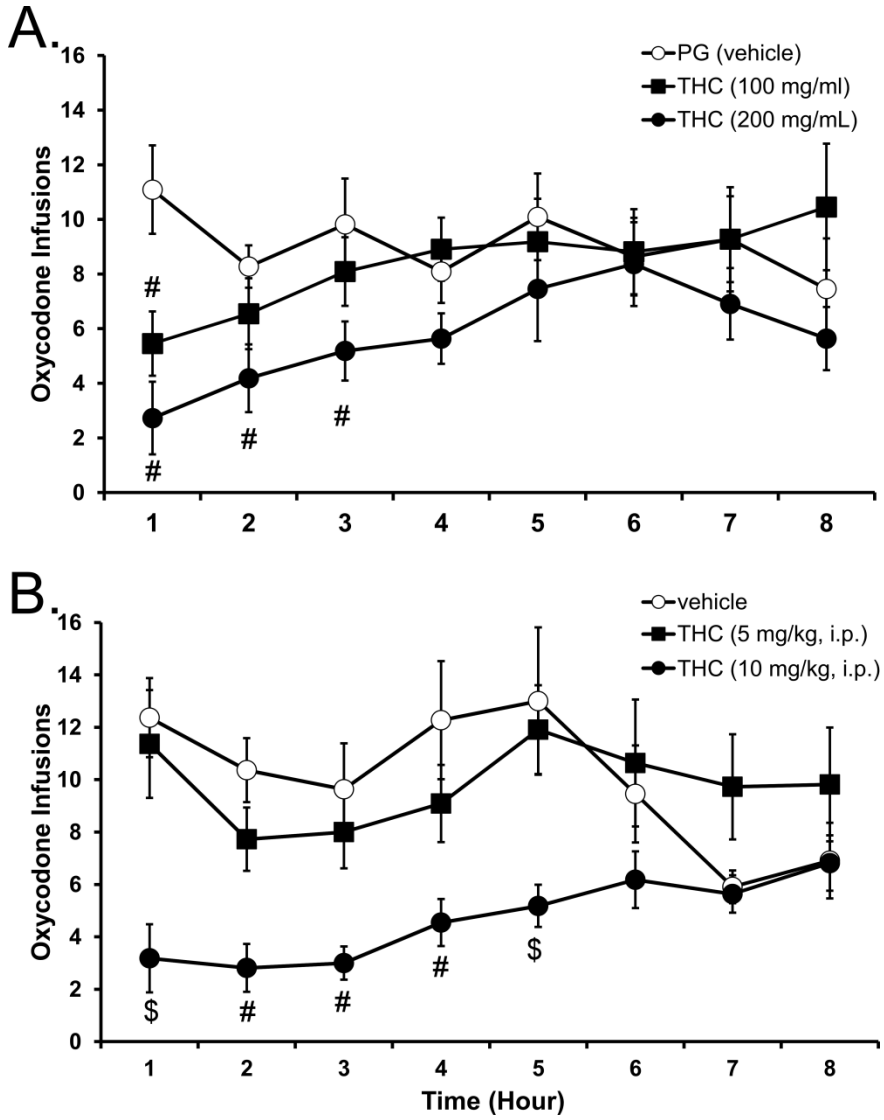

Figure 3. *THC vapor inhalation reduces oxycodone intake in a dose-dependent manner. Mean (N=11; +SEM) infusions for male rats trained to self-administer oxycodone following A) THC vapor (100,200 mg/ml) and B) injected THC (5,10 mg/kg, i.p.). Significant differences from vehicle condition are indicated by # and from all other dose conditions with $.*

difference in lever discrimination observed between PG and THC-exposed rats (76.59±3.85 and 73.92±7.33 percent, respectively).

Furthermore, the second study showed that inhalation of vaporized THC (100 and 200 mg/ml) significantly attenuated oxycodone self-administration (**Figure 3A**) compared to inhalation of PG vehicle and in a dose-dependent manner. In this study the vapor administration schedule was randomized for PG and 200 mg/mL on T, Th, with 100 mg/ml for all rats on the next F. Inhalation of 12.5 and 25 mg/ml THC (not shown) did not significantly decrease oxycodone self-administration. The rmANOVA confirmed significant main effects of Time [$F(7,70)=2.501$; $p<0.05$], of vapor Treatment [$F(2,20)=6.016$; $p<0.01$] and of the interaction of factors [$F(14,140)=2.322$; $p<0.01$]. Similarly, pre-session injection THC (0, 5, 10 mg/kg, i.p.) significantly reduced oxycodone intake (**Figure 3B**). The analysis confirmed significant effects of Time [$F(7,21)=2.729$; $p<0.01$], of vapor Treatment [$F(2,30)=7.168$; $p<0.01$] and of the Time x Treatment interaction [$F(30,210)=7.142$; $p<0.0001$]. The post hoc analysis further confirmed that oxycodone IVSA was significantly reduced for up to 5 h in rats pretreated with THC 10 mg/kg., i.p..

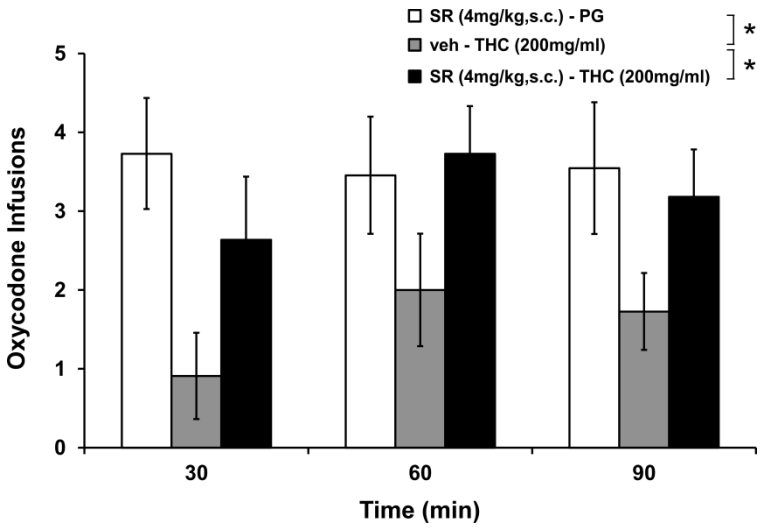

Figure 4. THC-mediated attenuation of oxycodone self-administration is CB_1 receptor-mediated. *Mean (N=11; ±SEM) infusions of oxycodone following vapor inhalation of THC and injection of CB_1 antagonist, SR141716 (SR; 4 mg/kg, i.p.) prior to the inhalation session. A significant difference between treatment conditions (across time bins) is indicated with *.*

THC vapor attenuates oxycodone self-administration via CB1 receptor activation

THC vapor-induced reduction of oxycodone IVSA was blocked by systemic administration of CB_1 antagonist,SR-141716 (4 mg/kg, i.p.; SR) prior to the vapor inhalation session (**Figure 4**). The ANOVA confirmed a significant main effect of Treatment [$F(2,20)=7.829$; $p<0.01$] and post hoc analysis of the marginal means confirmed that oxycodone intake following veh-THC pre-treatment was significantly lower than after either SR-PG or SR-THC pre-treatment conditions.

Mu opioid receptor agonism and antagonism

Experiments were conducted to determine the effects of pre-treating animals with mu opioid receptor (MOR) agonist (oxycodone) or MOR antagonist (naloxone) compounds. Pretreatment with oxycodone (0, 0.5, 1, 2 mg/kg, i.p.) significantly attenuated oxycodone IVSA in a dose-dependent manner (**Figure 5A**) and statistical analysis of the first 2 h confirmed a significant effect of oxycodone Treatment [$F(3,18)=8.401$; $p<0.01$]. Post hoc analysis of the marginal means confirmed that oxycodone intake after the highest pre-treatment dose (2.0 mg/kg, s.c.) was significantly lower than all other conditions.

Pretreatment with the mu opioid antagonist naloxone increased oxycodone self-administration (**Figure 5B**). The ANOVA confirmed significant effects of Treatment [$F(3,23)=4.128$; $p<0.05$], of Time [$F(3,69)=13.35$; $p<0.0001$], and of the interaction [$F(9,69)=2.9$; $p<0.01$] and the post hoc analysis further confirmed that significantly more infusions were obtained after 0.03 mg/kg naloxone, i.p., compared with all other treatment conditions from 30-60 minutes and compared with the 0.3 mg/kg pretreatment at 90 minutes.

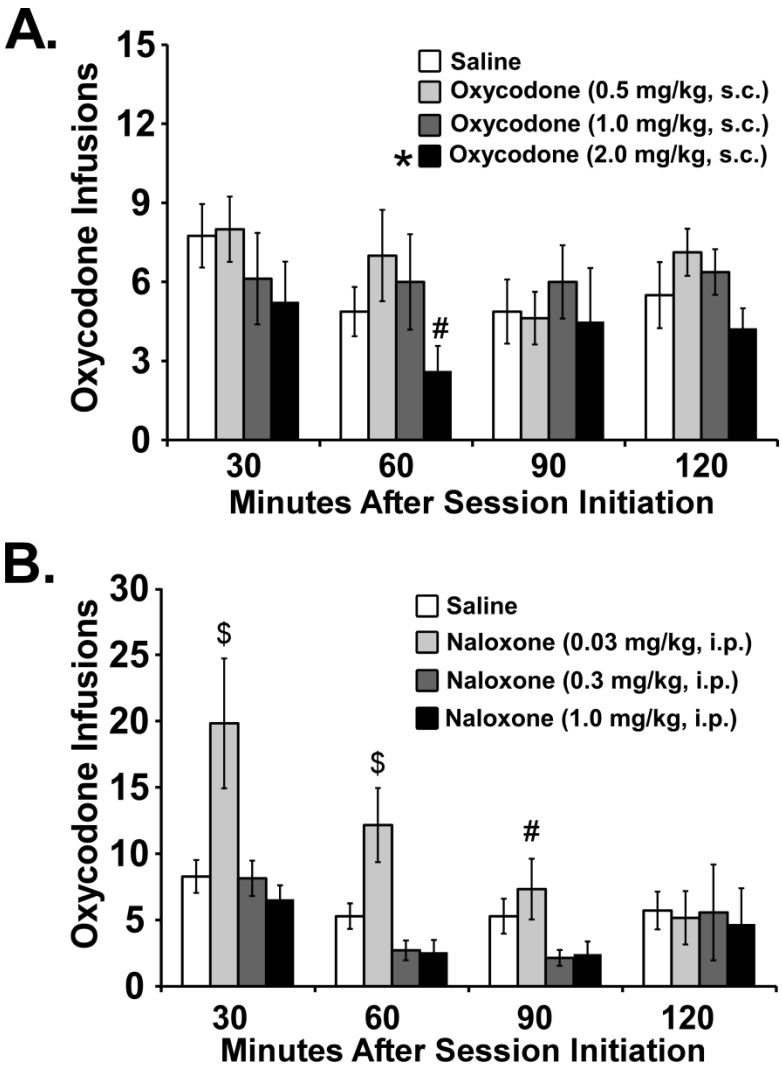

Figure 5. Mu opioid receptor agonists attenuate oxycodone intake. *Mean (+SEM) infusions of oxycodone following injection of A) oxycodone (N=8; 0.5-2 mg/kg, s.c.) or B) naloxone (N=6; 0.03-1.0 mg/kg, i.p.). A significant difference from the first hour time point is indicated by *. A significant difference from all other dose conditions is indicated with $ and a difference from the 0.3 is indicated with #.*

THC enhances oxycodone-induced anti-nociception

A study was next conducted to determine if injection of a combination of oxycodone and THC would produce interactive effects on anti-nociception (**Figure 6A**). A group of male (N=6) and female (N=8) Wistar rats were injected with the cannabinoid vehicle or THC (10 mg/kg, i.p.) 30 min prior to the saline vehicle or oxycodone (0.0 or 1 mg/kg, s.c.). The ANOVA confirmed significant effects of Time [F (3, 39) = 6.0; P<0.005], of Drug Condition [F(3,39)=41.47; *p*<0.0001], and of the interaction [F(9,117)=4.71; *p*<0.0001]. Post hoc analysis confirmed that THC or oxycodone administered alone failed to increase tail withdrawal latency; however, when THC and oxycodone administered in combination induced significantly higher tail withdrawal latency compared to other drug conditions at the 30 and 60 min time points. Similar effects were confirmed for the male (Time [F(3,15)=6.24; *p*<0.01]; Drug Condition [F(3,15)=44.42; *p*<0.0001]; Interaction [F(9,45)=2.76; *p*<0.05]; Post hoc: Combination > all other conditions 30-60 minutes post-injection) and female (Time [n.s.]; Drug Condition [F(3,21)=16.01; *p*<0.0001]; Interaction [F(9,63)=3.02; *p*<0.005] ; Post hoc: Combination > all other conditions 30-90 minutes post-injection) subgroups.

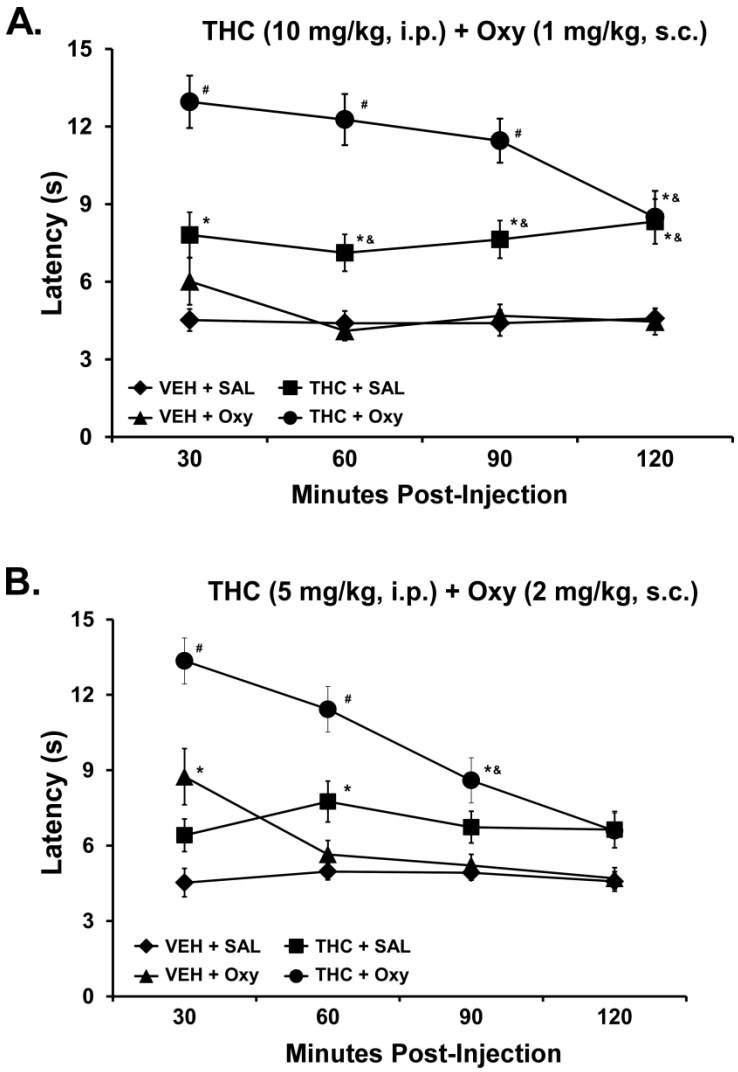

Figure 6. THC and oxycodone co-administration produces additive effects on antinociception. *A) Mean (N=14, 8F; ±SEM) tail withdrawal latency following administration of THC (10 mg/kg, i.p.), oxycodone (1 mg/kg, s.c.) or the combination. B) Mean (N=14, 8F; ±SEM) tail withdrawal latency following administration of THC (5 mg/kg, i.p.), oxycodone (2 mg/kg, s.c.) or the combination. Significant difference from all other treatments is indicated with #, a significant difference from VEH+Sal with * and a significant difference from VEH+Oxycodone with &. VEH= 1:1:8 vehicle used for THC; Sal = Saline vehicle used for oxycodone.*

The male and female rats were next injected with the vehicle or THC (5 mg/kg, i.p.) 30 min prior to the saline vehicle or oxycodone (0.0 or 2 mg/kg, s.c.) again in a randomized order (**Figure 6B**). The ANOVA confirmed significant effects of Time [F(3,156)=20.55; *p*<0.0001], of Drug Condition [F(3,52)=17.93; *p*<0.0001], and of the interaction [F(9,156)=9.21; *p*<0.0001]. Post hoc analysis confirmed that oxycodone administered alone significantly increased tail withdrawal latency (30 min), whereas the THC and oxycodone combination significantly increased latency compared to vehicle for up to 90 min. The additive effect of the combination of THC and oxycodone was significantly higher than the latency following THC (30 and 60 minutes) and oxycodone (30-90 minutes) conditions alone. Similar effects were confirmed for the male (Time [F(3,60)=15.66; *p*<0.0001]; Drug Condition [F(3,20)=13.5; *p*<0.0001]; Interaction [F (9, 60) = 4.7; P<0.0001]; Post hoc: Combination > all other conditions 60 minutes post-injection) and female (Time [F(3,84)=6.83; *p*<0.0005]; Drug Condition [F(3,28)=10.37; *p*<0.0001]; Interaction [F(9,84)=5.07; *p*<0.0001] ; Post hoc: Combination > all other conditions 30 minutes post-injection) subgroups.

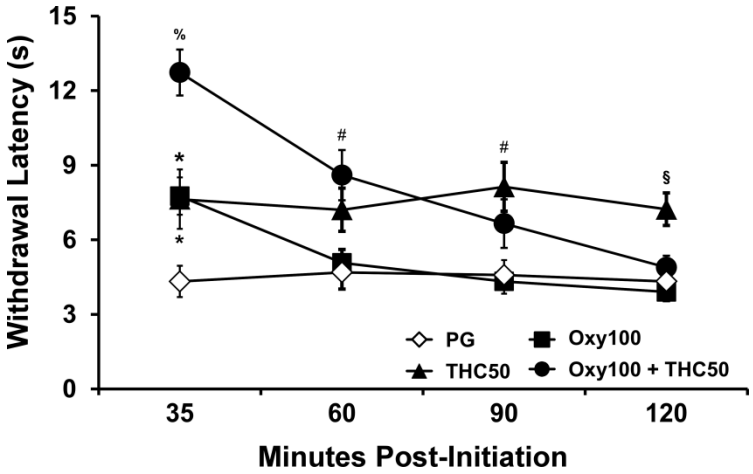

Figure 7. THC and oxycodone co-inhalation produces additive effects on antinociception. *Mean (N=10; ±SEM) tail withdrawal latency following inhalation of vapor from PG, THC (50 mg/mL), Oxycodone (100 mg/mL) or the THC/Oxycodone combination. A significant difference from PG and Oxy alone is indicated with #, a significant difference from all other conditions with %, a significant difference from PG with*, and a significant difference from Oxy alone with §.*

A separate group of rats (N=10) was tested for the anti-noceptive effects of vaporized oxycodone and THC. The tail withdrawal latency increased in rats following inhalation of vaporized oxycodone (100 mg/ml), THC (50 mg/ml) or the oxycodone:THC combination (**Figure 7**). The ANOVA confirmed significant main effects of Time after vapor initiation [F(3,27)=11.31; *p*<0.0001], of Drug Condition [F(3,27)=20.78; *p*<0.0001] and of the interaction of factors [F(9,81)=7.55; *p*<0.0001]. The post hoc analysis confirmed that inhalation of combined oxycodone and THC significantly increased tail

withdrawal latency compared to PG (35-60 minutes after vapor initiation), oxycodone alone (35-60 minutes after vapor initiation) or THC alone (35 minutes after vapor initiation). Significantly increased latency compared with PG inhalation was also observed after inhalation of oxycodone alone (35 minutes after vapor initiation) or THC alone (35,90 minutes after vapor initiation).

Discussion

This study found that THC interacts with the effects of oxycodone when the two drugs are co-administered such that THC enhances the effects of a given dose of oxycodone, *in vivo*. This manifested both in a reduction in the amount of oxycodone that was self-administered under either short or extended daily access conditions, and an increase in the magnitude and duration of anti-nociception produced by oxycodone. It is particularly notable that THC reduced the self-administration of oxycodone even in rats trained to escalated intake levels using an extended access (8 h) paradigm. These effects were lasting, since a single THC delivery by injection or inhalation significantly reduced the self-administration of oxycodone for up to 5 hours. The impact of THC was dose-dependent and it generalized across the injection and inhalation routes of administration. A prior investigation showed that THC-induced hypothermia lasts at least 6 h after injection of 10 mg/kg, i.p., but only about 2 h after vapor inhalation for 30 minutes (Nguyen *et al*, 2016b; Taffe *et al*, 2014). Thus it may be that inhaled THC can reduce oxycodone use with fewer side effects compared with an equivalently effective THC dose delivered by a less rapid route of administration. THC-mediated enhancement of the anti-nociceptive effects of oxycodone were likewise present across both routes of administration

In addition to the dose-dependency of the effect of THC on oxycodone IVSA, an interpretation of mechanistic specificity is further enhanced by the finding that prior administration of the CB_1 antagonist SR 141716 blocked the effects of THC inhalation. These data can be considered with the effects of mu opioid receptor agonist and agonist pretreatment on the IVSA of oxycodone in which the agonist oxycodone decreased, whereas the mu opioid antagonist naloxone increased, the amount of oxycodone that was self-administered. This supports the conclusion that the mechanism by which THC decreases oxycodone IVSA is mediated by the CB_1 receptor and produces an enhancement of the effects of

oxycodone at the mu opioid receptor. Opposing the effects of oxycodone would increase, rather than decrease, self-administration as was found after naloxone pre-treatment.

The Oxy-TT vaccinated animals self-administered more oxycodone under FR1 response contingency conditions and about the same as the controls under a PR contingency during the acquisition period. This is consistent with two similarly vaccinated groups in a prior finding (Nguyen *et al*, 2017b) and is likely a behavioral marker of the ~50% decrease in brain oxycodone that is produced. In the present study, the relative impact of THC inhalation to suppressed IVSA was similar in each group and, if anything, slightly lesser in the Oxy-TT group. This outcome is also consistent with an effect of THC on the rewarding value of self-administered oxycodone rather than a general behavioral suppression.

The study also showed that sensation for a noxious stimulus, as classic preclinical model of analgesic activity, was additively diminished by the co-administration of THC with oxycodone compared with either drug administered alone. Prior work has shown anti-nociceptive interactions between mu-opioid and cannabinoid receptor ligands in formalin test of inflammatory pain (Yuill *et al*, 2017) and in nociception rhesus monkeys (Li *et al*, 2008) as reviewed above. The present study extends those results to the interaction of THC with the effects of the prescription opioid, oxycodone. It was interesting that the interactive anti-nociceptive effects of THC with oxycodone appeared to last long past the duration of activity of oxycodone administered by itself, which was only about 30 minutes. This may suggest a second benefit of adding THC to oxycodone (i.e., extended duration of action) in addition to the primary effect, (i.e., a reduction of dose to produce comparable immediate effect).

In conclusion this study confirmed additive effects of THC and oxycodone within preclinical models of both oxycodone reward and analgesia. This provides additional experimental evidence for the likely pharmacological specificity of epidemiological findings, i.e. from medical marijuana states. These data suggests that co-use of marijuana and prescriptions opioids such as oxycodone might provide effective pain control with lower doses than would be required for either drug alone. There was also evidence that THC can attenuate the self-administration of oxycodone, potentially suggesting a therapeutic effect for those attempting to reduce non-medical oxycodone use. Thus, further investigation

of cannabinoid / opioid interactions may identify improved therapeutic approaches for analgesia and possible mechanisms to reduce opioid addiction.

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