ORIGINAL INVESTIGATION

Effects of combined THC and heroin vapor inhalation in rats

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Abstract

Rationale Opioids are effective medications, but they have several key limitations including the development of tolerance, establishment of dependence, diversion for non-medical use, and the development of addiction. Therefore, any drugs which act in an additive or synergistic fashion with opioids to address medical applications have the potential to reduce opioid-related harms.

Objectives To determine if heroin and Δ^9-tetrahydrocannabinol (THC) interact in an additive or independent manner to alter nociception, body temperature, and spontaneous locomotor activity when inhaled or injected.

Methods Groups of female and male rats, implanted with radiotelemetry transmitters, were exposed to vapor generated from heroin (50 mg/mL in propylene glycol vehicle; PG), THC (50 mg/mL), or the combination for assessment of effects on temperature and activity. Thermal nociception was assessed with a warm water tail-withdrawal assay.

Results Heroin inhalation increased temperature and activity whereas THC inhalation decreased temperature and activity in both female and male Sprague–Dawley rats. Effects of combined inhalation were in opposition, and additional experiments found the same outcome for the injection of heroin (0.5 mg/kg, s.c.) and THC (10 mg/kg, i.p.) alone and in combination. In contrast, the co-administration of heroin and THC by either inhalation or injection produced additive effects on thermal nociception in both male and female Sprague–Dawley and Wistar rats.

Conclusions This study shows that additive effects of THC with an opioid on a medical endpoint such as analgesia may not generalize to other behavioral or physiological effects, which may be a positive outcome for unwanted side effects.

Keywords e-cigarette · Vape · Opioid · Nociception · Locomotor activity · Thermoregulation

Introduction

Expansion of the use of cannabis for purported medical benefits (e.g., for pain) stimulates interest in the possible opioid-sparing effects of cannabis constituents, including the primary psychoactive compound Δ^9-tetrahydrocannabinol (THC). While opioids are effective medications, they have many limitations including the development of tolerance (and the consequent need to increase the doses to obtain

similar efficacy), the development of dependence (which counter-indicates rapid discontinuation), diversion for non-medical use, and the development of addiction. Any drugs which may act in an additive or synergistic fashion with opioids therefore have the potential to reduce opioid-related concerns. There are known interactions by which endogenous cannabinoid receptor 1 (CB$_1$) ligands may enhance signaling of mu opioid receptors (Parsons and Hurd 2015), thus generating a reasonable mechanistic hypothesis for evaluating opioid-sparing effects of cannabis constituents.

Cannabis co-use triples the risk of dependence on heroin in those diagnosed with a substance use disorder (Crummy et al. 2020) and cannabis users over 50 years of age exhibit a 6.3 increased odds ratio of heroin use (Ramadan et al. 2020). Adolescents in one urban setting who use cannabis regularly were at twice the risk for opioid misuse compared with occasional users of cannabis (Reboussin et al. 2020) and age 14 onsets of frequent cannabis use increased the risk of

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opioid use at age 19 (Thrul et al. 2021). Almost two-thirds of individuals in one sample first used heroin while co-using cannabis (Olthuis et al. 2013) and 50–60% of individuals in heroin- and methadone-maintenance treatment for opioid use disorder were co-using cannabis (Musshoff et al. 2010). On a day-by-day basis, cannabis use doubles the risk of non-medical opioid use in adults with problematic substance use (Gorfinkel et al. 2021). Thus, although cannabis may have the potential to reduce opioid use in medical patients, there is also a clear risk for cannabis to increase the non-medical use of heroin from adolescence into middle age. Consideration of these phenomena spurs interest in determining the interactive effects of cannabinoids and opioids across multiple behavioral and physiological endpoints to lend greater context for "opioid-sparing" recommendations for cannabis use.

We recently presented evidence that THC enhances the effects of oxycodone in an anti-nociception assay in rats and it also enhances the effects of a unit dose of oxycodone or heroin when self-administered (Nguyen et al. 2019). Maguire and France have shown that the nature of the anti-nociceptive interaction (supra-additive, sub-additive, additive) between cannabinoids and opioids may depend on the specific drugs that are involved (Gerak et al. 2019; Maguire and France 2018; Maguire et al. 2013) which cautions against making generalizations across either class of substances, before specific data are available. In the case of both nociception and drug self-administration, the effects of opioids and cannabinoids are often in the same direction, i.e., anti-nociceptive (Li et al. 2008; Lichtman and Martin, 1990; Manning et al. 2001; Peckham and Traynor 2006; Wakley and Craft 2011) and rewarding (Blakesley et al. 1972; Justinova et al. 2003; Killian et al. 1978; Panlilio et al. 2010; Vendruscolo et al. 2018). This can make it difficult to determine if the outcome of co-administration is due to the additive effects of independent mechanisms or the interaction of signaling within the same mechanistic pathways (Ahmad et al. 2013).

Determination of any heroin/THC interactions for in vivo endpoints that are expected to change in *opposite* directions after each drug is administered individually can help to further parse the specificity of any apparent additive effects. We have identified conditions under which either inhaled or injected heroin can *increase* the body temperature and spontaneous locomotor activity (Gutierrez et al. 2021), and conditions under which THC can *decrease* body temperature and locomotor activity (Javadi-Paydar et al. 2018; Nguyen et al. 2016b; Taffe et al. 2021). We have further shown that the locomotor effects of nicotine and THC on activity can oppose each other when co-administered (Javadi-Paydar et al. 2019b); the effects of each drug to decrease body temperature were also dissociated across time, after administration. This study was conducted to determine if heroin and THC interact in an additive or independent manner to alter thermal nociception, body temperature, or spontaneous locomotor activity when inhaled or injected.

The recent broad availability of e-cigarette style Electronic Drug Delivery Systems (EDDS) supports the possibility of delivering a range of drugs other than nicotine, including opioids, via vapor inhalation. The use of these devices for the ingestion of cannabis extracts has become increasingly popular (Allem et al. 2019; Dugas et al. 2020; Kowitt et al. 2019; Nicksic et al. 2020; Pearson and Villanti, 2020). The EDDS can be used to deliver active doses of a wide range of drugs including amphetamine and cathinone derivative psychomotor stimulants (Nguyen et al. 2016a, 2017), opioids (Gutierrez et al. 2020a; Moussawi et al. 2020; Nguyen et al. 2019; Vendruscolo et al. 2018), cannabinoids (Breit et al. 2020; Freels et al. 2020; Javadi-Paydar et al. 2019a, 2018; Moore et al. 2020a; Nguyen et al. 2016b), and nicotine (Cooper et al. 2021; Frie et al. 2020; Javadi-Paydar et al. 2019b; Montanari et al. 2020; Ponzoni et al. 2015) to rats and mice; for review see (Miliano et al. 2020; Moore et al. 2020b). Generally speaking, the effects of drugs after inhalation persist for a shorter time than when injected (i.p. or s.c.), and, as we have recently shown, this is certainly true for heroin and THC (Gutierrez et al. 2021; Nguyen et al. 2016b). This difference may impact the combined effects, and therefore, the inhalation route was contrasted with traditional injection routes used in rodent models.

Methods

Subjects

Male ($N=15$) and female ($N=7$) Sprague–Dawley rats (Harlan/Envigo, Livermore, CA) and male ($N=10$) and female ($N=22$) Wistar (Charles River) rats were housed in humidity- and temperature-controlled (23 ± 2 °C) vivaria on 12:12-h (reversed) light:dark cycles. Rats 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 or the University of California, San Diego.

Drugs

Heroin (diamorphine HCl) and Δ^9-tetrahydrocannabinol (THC) were administered by vapor inhalation with doses described by the concentration in the propylene glycol (PG) vehicle (e.g., 50, 100 mg/mL) and duration of inhalation (15, 30 min), following the methods described in prior studies (Javadi-Paydar et al. 2018; Nguyen et al. 2016b). Heroin was also administered subcutaneously. THC was

also administered intraperitoneally in a dose of 10 mg/kg, which produces robust temperature responses (Nguyen et al. 2016b; Taffe et al. 2015). Naloxone was administered i.p. For injection, heroin and naloxone were dissolved in physiological saline and THC was suspended in a vehicle comprised of ethanol:cremulphor:saline in a 1:1:18 ratio. The THC and heroin were provided by the US National Institute on Drug Abuse Drug Supply Program.

Nociception

Effects on nociception were assessed using a warm-water tail immersion assay, as described in Javadi-Paydar et al. (2018) and Nguyen et al. (2018a, 2018b). Briefly, the tail was inserted ~3–4 cm into a warm (52 °C) water bath and the latency for tail removal recorded with a stopwatch. A 15-s cutoff was used to avoid any potential for tissue damage.

Inhalation apparatus

Vapor was delivered into sealed vapor exposure chambers (152 mm W × 178 mm H × 330 mm L; La Jolla Alcohol Research, Inc, La Jolla, CA, USA) through the use of e-vape controllers (Model SSV-3 or SVS-200; 58 watts, 0.24–0.26 ohms, 3.95–4.3 V, ~214 °F; La Jolla Alcohol Research, Inc, La Jolla, CA, USA) to trigger Smok Baby Beast Brother TFV8 sub-ohm tanks. Tanks were equipped with V8 X-Baby M2 0.25 Ω coils. MedPC IV software was used to schedule and trigger vapor delivery (Med Associates, St. Albans, VT USA). The apparatus and settings were the same for all drug conditions. 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. Airflow was initiated 30 s prior to, and discontinued 10 s after, each puff.

Radiotelemetry

Female ($N = 7$) and male ($N = 15$) Sprague–Dawley rats implanted with sterile radiotelemetry transmitters (Data Sciences International, St Paul, MN) in the abdominal cavity as previously described (Taffe et al. 2015; Wright et al. 2012) were used in this investigation. For studies, animals were evaluated in clean standard plastic homecages (~1 cm layer of sani-chip bedding) in a dark testing room, separate from the vivarium, during the (vivarium) dark cycle. Radiotelemetry transmissions were collected via telemetry receiver plates (Data Sciences International, St Paul, MN; RPC-1 or RMC-1) placed under the cages as described in prior investigations (Aarde et al. 2013; Miller et al. 2013). Test sessions

for inhalation and injection studies started with a 15-min interval to ensure data collection, then a 15-min interval for baseline temperature and activity values followed by the initiation of vapor sessions or drug injection.

Experiments

Experiment 1 (inhalation dose ranging in male Sprague–Dawley rats)

A preliminary dose-ranging experiment was conducted in a group ($N = 8$) of male Sprague Dawley rats used previously in investigations of the effects of inhalation of cannabidiol, nicotine, and THC as reported (Javadi-Paydar et al. 2019a, 2019b). The first goal was to evaluate whether heroin by vapor inhalation would alter body temperature and spontaneous locomotion, as it does when injected. These were our first attempts, conducted prior to the subsequent investigations reported here and in prior publications (Gutierrez et al. 2021, 2020b), critical to establishing the efficacy of drug delivery by this method. The second goal was to identify sub-maximal exposure conditions such that interactions with another drug such as THC might be detected. All animals were habituated to the procedure with one session of 30-min vapor exposure to PG followed by a 60-min recording interval. To determine the effect of heroin vapor on body temperature and locomotor activity, an initial 30-min inhalation exposure to PG and Heroin (50, 100 mg/mL in the PG) was designed. Animals were randomized to treatment conditions, and exposed, in pairs. Subsequently, the animals were evaluated after a 15-min interval of exposure to PG, heroin (50 mg/mL), THC (50 mg/mL), or heroin (50 mg/mL) + THC (50 mg/mL). The treatment conditions were again counter-balanced across exposure pairs and THC was experienced no more frequently than once per week for a given rat.

Experiment 2 (inhalation in male and female Sprague–Dawley rats)

Groups ($N = 7$) of male and female Sprague–Dawley rats used in studies previously reported (Gutierrez et al. 2021) were recorded for a baseline interval, exposed to inhalation of vapor from PG, heroin (50 mg/mL in the PG), THC (50 mg/mL), or the combination (50 mg/mL for each drug) for 30 min in groups of 2–3 per exposure. These studies were conducted subsequent to a series of similar challenges with vapor inhalation of nicotine, THC, and with an injection of THC in additional studies not previously reported. The tail-withdrawal assay (experiment 4, below) was conducted immediately after the vapor session and then rats were returned to their individual recording chambers for telemetry assessment. The four inhalation conditions were

evaluated in a counterbalanced order across pairs/triplets, no more frequently than every 3–4 days.

Experiment 3 (injection in male and female Sprague–Dawley rats)

The groups ($N = 7$) of male and female Sprague–Dawley rats used in experiments 2 and 4 were recorded for a baseline interval, and then injected with THC (10 mg/kg, i.p.) and saline (s.c.), the 1:1:18 vehicle (i.p.) and heroin (0.5 mg/kg, s.c.), THC (10 mg/kg, i.p.) and Heroin (0.5 mg/kg, s.c.), or the 1:1:18 vehicle and saline. For this study, treatments were twice per week (3–4-day intervals), counterbalanced across individuals, albeit with a minimum 7-day interval between any THC injections.

Experiment 4 (nociception in male and female Sprague–Dawley rats)

A nociception assay was conducted immediately after the vapor sessions during experiment 2, and then rats were returned to their individual recording chambers for the telemetry assessment. The latency to withdraw the tail from insertion (3–4 cm) into a 52 °C water bath was recorded by stopwatch, using techniques previously reported (Gutierrez et al. 2020b; Javadi-Paydar et al. 2018; Nguyen et al. 2019). A 15-s cutoff was used as the maximal latency for this assay to avoid any potential for tissue damage.

Experiment 5 (naloxone antagonism in male Sprague–Dawley rats)

An opioid receptor antagonist study was included because we had not included this evidence in a prior study reporting heroin vapor effects on similar endpoints (Gutierrez et al. 2021) and we have reported an unusual lack of effect of cannabinoid receptor 1 (CB_1) antagonist/inverse agonist pre-treatment on THC vapor-induced hypothermia (Nguyen et al. 2020). This latter was observed despite efficacy against the anti-nociceptive effects of inhaled THC and the thermoregulatory effects in injected THC; thus, it was of interest to determine if a nonselective opioid receptor antagonist was effective against the effects of inhaled heroin on temperature, activity, and anti-nociceptive responses. The group ($N = 7$) of male Sprague–Dawley rats used in experiments 2–4 was recorded for a baseline interval, and then injected with either saline or naloxone (1.0 mg/kg, i.p.) 15 min prior to the start of a 30-min inhalation session of either PG or Heroin (100 mg/mL) vapor. Post-inhalation, a tail-withdrawal assay was conducted (as in experiment 4) and then animals were returned to their recording chambers for telemetric assessment. Inhalation was conducted in two pairs and one triplet, with the groupings changed on each test day. Pre-treatments

were varied within the inhalation groupings to provide further randomization of conditions. Sessions were conducted two times per week (3–4-day interval) in a counterbalanced treatment order. The female group examined in parallel with these animals in prior studies was not included because the maximum number of treatment days under the approved protocol had been used in prior experiments.

Experiment 6 (anti-nociception in male and female Wistar rats)

Groups of female ($N = 12$) and male ($N = 10$) Wistar rats were used in these studies. Rats had been exposed to twice daily sessions (30 min) of vapor from the propylene glycol (PG) vehicle or heroin (100 mg/mL in the PG) from PND 36 to PND 45 (Gutierrez et al. 2020a). These anti-nociception experiments were conducted between PND 253 and PND 275. Four treatment conditions were evaluated in counter-balanced order. Each session started with a tail-withdrawal assessment before any injections (pre-treatment). This was followed immediately with an injection of THC (10 mg/kg, i.p.) or the cannabinoid vehicle (1:1:18 ethanol:cremulphor:saline). Another tail-withdrawal assessment was conducted 30 min post-injection, followed by a second injection of either heroin (0.5 mg/kg, s.c.) or saline (the vehicle for heroin), and then a final tail-withdrawal 30 min later. We have shown previously that anti-nociceptive effects of THC when injected at 5 or 10 mg/kg, i.p., persist essentially unchanged from 30 to 90 min after injection (Nguyen et al. 2019), justifying this sequential assessment approach. The design resulted in four replications of the pre-treatment baselines, two replications of the THC or vehicle condition and then four final conditions of vehicle-saline, vehicle-heroin, THC-saline, and THC-heroin.

Experiment 7 (anti-nociception in experimentally naive female Wistar rats)

A group ($N = 10$) of female Wistar rats were used in these studies. Rats entered the laboratory at 11 weeks of age but due to laboratory slowdowns associated with the 2020 COVID-19 crisis remained experimentally naïve for 29 weeks. Therefore, this group was an experimentally naïve group that was well age matched with the female rats used in experiment 5. The goal of this study was to better determine the threshold for a heroin dose which would interact with a minimal, but effective, dose of THC. Animals were first assessed for tail-withdrawal latency at baseline (no injection) or 30 min after heroin (0.0, 0.1, 0.32, 0.56, 1.0 mg/kg, s.c.) to better determine the lower end of the dose–effect function. For this experiment, two conditions were assessed each day with 2 h between each injection and 1 week between assessment days. Three within-day orders were assessed in

a counterbalanced order by cage dyad, including baseline saline, 0.1–0.56, and 0.32–1.0. In the next experiment, rats were assessed for tail withdrawal three times in a day including a pre-treatment baseline, 30 min after injection of either the 1:1:18 cannabinoid vehicle or 5.0 mg/kg THC, i.p. and then 30 min after injection of heroin (0.0, 0.1, 0.32, 0.56 mg/kg, s.c.). Thus, the design produced 8 total treatment conditions defined by the four heroin doses and the injection of THC or its vehicle before the heroin.

Data analysis

The telemeterized body temperature and activity rate (counts per minute) were collected on a 5-min schedule in telemetry studies but are expressed as 15-min (initial dose range study) or 30-min averages for primary analysis. The time courses for data collection are expressed relative to the initiation of vapor inhalation and times in the figures refer to the initiation of the interval (e.g. "90 min" reflects the data collected on the six sequential 5-min intervals from 65 to 90 min, inclusively). Due to transfer time after vapor sessions, the "60-min" interval reflects the average of collections from ~40 to 60 min). Any missing temperature values were interpolated from the values before and after the lost time point. Activity rate values were not interpolated because 5 to 5-min values can change dramatically; thus, there is no justification for interpolating. Data (activity rate, body temperature, tail-withdrawal latency) were generally analyzed with two-way analysis of variance (ANOVA) including repeated measures factors for the drug treatment condition and the time after vapor initiation or injection. A third factor was used for pre-treatments, adolescent exposure group, or sex in some experiments as described in the "Results" section. A mixed-effects model was used instead of ANOVA wherever there were missing datapoints. Any significant main effects were followed with post hoc analysis using Tukey (multi-level factors) or Sidak (two-level factors) procedures. The criterion for inferring a significant difference was set to $p < 0.05$. All analyses used Prism 8 or 9 for Windows (v. 8.4.2 or 9.0; GraphPad Software, Inc, San Diego, CA).

Results

### Experiment 1 (inhalation dose ranging in male Sprague–Dawley rats)

The first 30-min inhalation duration study was originally designed to include a heroin (100 mg/mL) condition; however, the first two animals randomized to this condition were substantially sedated. This condition was therefore discontinued for this experiment and these two animals were omitted from the rest of this study. Significant effects (Fig. 1A, C)

of time post-initiation and the interaction of time with vapor condition were confirmed for both temperature (time: $F_{(8, 40)} = 3.48$ $P < 0.005$; interaction: $F_{(8, 40)} = 4.17$; $P < 0.005$) and activity rate (time: $F_{(8, 40)} = 10.34$; $P < 0.0001$; interaction: $F_{(8, 40)} = 4.48$; $P < 0.001$). The post hoc test confirmed that both temperature and activity were significantly lower after heroin (50 mg/mL for 30 min) inhalation compared with the pre-inhalation baseline and with the PG inhalation condition in the interval 45–60 min after the start of inhalation.

In the 15-min exposure study (Fig. 1B, D), the ANOVAs confirmed that there were significant effects of time post-initiation ($F_{(9, 63)} = 11.04$; $P < 0.0001$), vapor condition ($F_{(3, 21)} = 17.75$; $P < 0.0001$), and the interaction of time with vapor condition ($F_{(27, 189)} = 7.09$; $P < 0.0001$) on temperature and significant effects of time ($F_{(9, 63)} = 11.60$; $P < 0.0001$) and the interaction of time with vapor condition ($F_{(27, 189)} = 2.60$; $P < 0.0001$) on activity rate. The post hoc test further confirmed that body temperature was higher after heroin inhalation (30–105 min after the start of exposure), and lower in the THC condition (30–105, 120–135 min), compared with the PG condition; temperature likewise differed between THC and heroin conditions (30–150 min). Temperature was also higher in the heroin condition (30–60 min) and lower in the THC condition (30–120 min) in comparison with temperature in the THC+heroin combined condition. The post hoc exploration also confirmed that activity was lower in the heroin (30 min) and THC conditions (105–135 min) compared with the PG condition. Significantly lower activity was also confirmed relative to the THC+heroin condition for heroin (30 min) and THC (75–90 min) and activity differed between THC and heroin conditions (30, 75–105 min).

Experiment 2 (inhalation in male and female Sprague–Dawley rats)

The body temperature of the female rats (Fig. 2A) was significantly elevated by the inhalation of heroin 50 mg/mL and decreased by the inhalation of THC (50 mg/mL). The ANOVA confirmed a significant effect of time after vapor initiation ($F_{(8, 48)} = 25.65$; $P < 0.0001$) of vapor condition ($F_{(3, 18)} = 43.71$; $P < 0.0001$) and of the interaction of time with vapor condition ($F_{(24, 144)} = 16.13$; $P < 0.0001$) on body temperature. The post hoc test confirmed that temperature was significantly higher after inhalation of heroin 50 mg/mL (90–120 min after the start of inhalation) and lower after inhalation of THC 50 mg/mL (60–270 min) compared with the PG condition. Temperature following the inhalation of both drugs in combination was significantly lower compared with PG (60–90, 210–270 min) and heroin (60–210 min) and higher compared with THC (60–180 min). Temperature was also significantly lower than the baseline
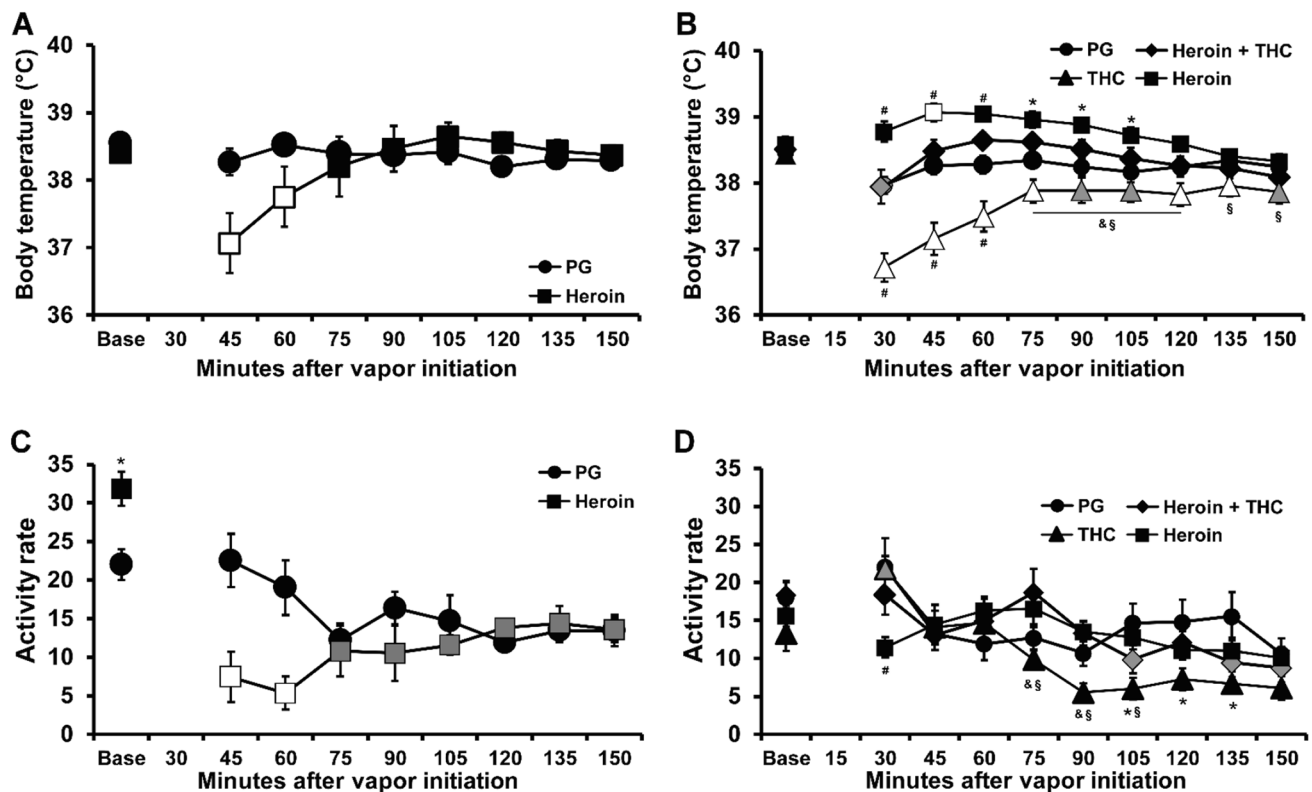
Fig. 1 Mean body temperature (**A**) and activity rate (**C**) for male Sprague–Dawley rats ($N=6; \pm$ SEM) after vapor inhalation of the propylene glycol vehicle (PG) or heroin (50 mg/mL in the PG) for 30 min. Mean body temperature (**B**) and activity rate (**D**) for male Sprague–Dawley rats ($N=8; \pm$ SEM) after vapor inhalation of the propylene glycol vehicle (PG), heroin (50 mg/mL in the PG), THC (50 mg/mL), or the combination (heroin+THC) for 15 min. Open symbols indicate a significant difference from both the vehicle at a given time-point and the within-treatment baseline, while shaded symbols indicate a significant difference from the baseline, within treatment condition, only. A significant difference from the PG inhalation condition is indicated by an asterisk, a difference from the heroin condition with a section sign, a difference from the heroin+THC conditions with an ampersand, and from all other conditions with a number sign

observation 60–270 min after the inhalation of THC or the combination, but not in the other two vapor conditions.

The activity of the female rats (Fig. 2C) was increased after heroin inhalation and decreased after inhalation of the THC+heroin combination. The ANOVA confirmed a significant effect of time after vapor initiation ($F(8, 48)=9.81$; $P<0.0001$) of vapor condition (F $(3, 18)=6.97$; $P=0.0026$) and of the interaction of time with vapor condition ($F(24, 144)=3.91$; $P<0.0001$) on activity rate. The post hoc test confirmed that activity was decreased relative to the baseline and all other inhalation conditions 60 min after the start of THC+heroin inhalation and elevated relative to the baseline and all other inhalation conditions 60 min after the start of THC. Activity was elevated compared with all other conditions 90–120 min after the start of heroin inhalation.

The body temperature of the male rats (Fig. 2B) was also significantly elevated by the inhalation of heroin 50 mg/mL and decreased by the inhalation of THC (50 mg/mL). The ANOVA confirmed a significant effect of time after vapor initiation ($F(8, 48)=2.64$; $P<0.05$) of vapor condition (F

$(3, 18)=7.84$; $P=0.0015$) and of the interaction of time with vapor condition ($F(24, 144)=5.26$; $P<0.0001$) on body temperature. Temperature was significantly lowered after the inhalation of THC (60–210 min after the start of inhalation) and was significantly increased 60–90 min after the start of heroin inhalation, relative to the baseline observation, but was unchanged in the other two conditions. Similarly, the post hoc test confirmed that temperature was significantly higher after inhalation of heroin 50 mg/mL (90–120 min after the start of inhalation) and lower after inhalation of THC 50 mg/mL (60–90, 150–210 min) or the combination (120 min), compared with the PG condition. Temperature following the inhalation of both drugs in combination was significantly lower compared with heroin (90 min) and higher compared with THC (60–210 min).

The activity of the male rats was (Fig. 2D) was elevated after heroin inhalation, as confirmed with a significant effect of time after vapor initiation ($F(8, 48)=13.41$; $P<0.0001$) of vapor condition ($F(3, 18)=16.81$; $P<0.0001$) and of the interaction of time with vapor condition ($F(24, 144)=2.69$;
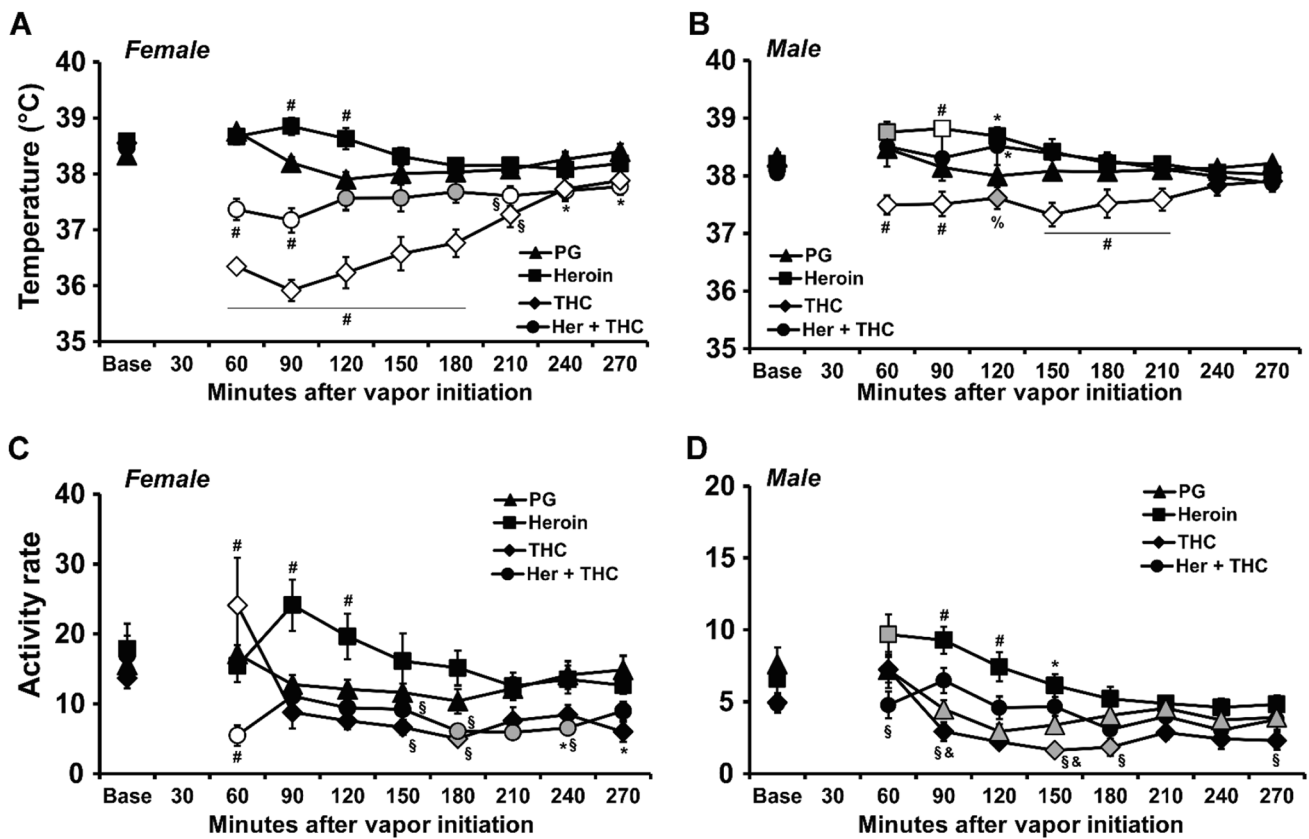
Fig. 2 Mean ($N=7$ per group; $\pm$ SEM) body temperature (**A**, **B**) and activity rate (**C**, **D**) for female (**A**, **C**) and male (**B**, **D**) Sprague–Dawley rats after vapor inhalation of the propylene glycol vehicle (PG), *her*oin (50 mg/mL in the PG), THC (50 mg/mL), or the combination of heroin and THC (at 50 mg/mL each) for 30 min. N.b. there is a twofold difference in the scale of the activity in **C** and **D**, to accommodate the sex difference in activity rate. Open symbols indicate a significant difference from both the vehicle at a given time-point and the within-treatment baseline, while shaded symbols indicate a significant difference from the baseline, within treatment condition, only. A significant difference from the PG inhalation condition is indicated by an asterisk, a difference from the heroin condition with a section sign, a difference from the THC condition with a percent sign, a difference from the heroin + THC conditions with an ampersand, and from all other conditions with a number sign

$P = 0.0001$) on activity rate in the ANOVA. The post hoc test confirmed that activity was elevated 90–120 min after the start of heroin inhalation compared with all other conditions, as well as at individual timepoints relative to the combination (60 min), THC (150, 180, 270 min), and PG (150 min). Activity differed between TH and the combined inhalation (90, 150 min) was higher than the baseline 60 min after initiation of heroin and lower than the baseline in the PG (90–270 min after initiation) and THC (150–180 min) inhalation conditions.

Experiment 3 (injection in male and female Sprague–Dawley rats)

The injection of THC (10 mg/kg, i.p.) and heroin (0.5 mg/kg, s.c.) produced opposing effects on activity in male and female groups and opposing effects on temperature in the male rats (Fig. 3). The body temperature of the female rats

was significantly decreased by THC (Fig. 3A). The ANOVA confirmed a significant effect of time after injection (F (9, 45) = 13.44; $P < 0.0001$) of drug treatment condition (F (3, 15) = 16.13; $P < 0.0001$) and of the interaction of time with drug condition (F (27, 135) = 7.14; $P < 0.0001$) on body temperature in the female rats. The post hoc test confirmed that temperature was changed relative to the baseline and also relative to the vehicle + saline and heroin injection conditions, after the injection of THC (30–270 min post-injection) or the combination (30–270 min).

The activity of the female rats was increased by heroin injection and decreased by the THC injection (Fig. 3B) as confirmed by a significant effect of time after injection (F (9, 54) = 9.47; $P < 0.0001$) of drug treatment condition (F (3, 18) = 17.61; $P < 0.0001$) and of the interaction of time with drug condition (F (27, 162) = 2.43; $P < 0.0005$) on activity in the ANOVA. The post hoc test confirmed that activity rates were reduced compared with the baseline and
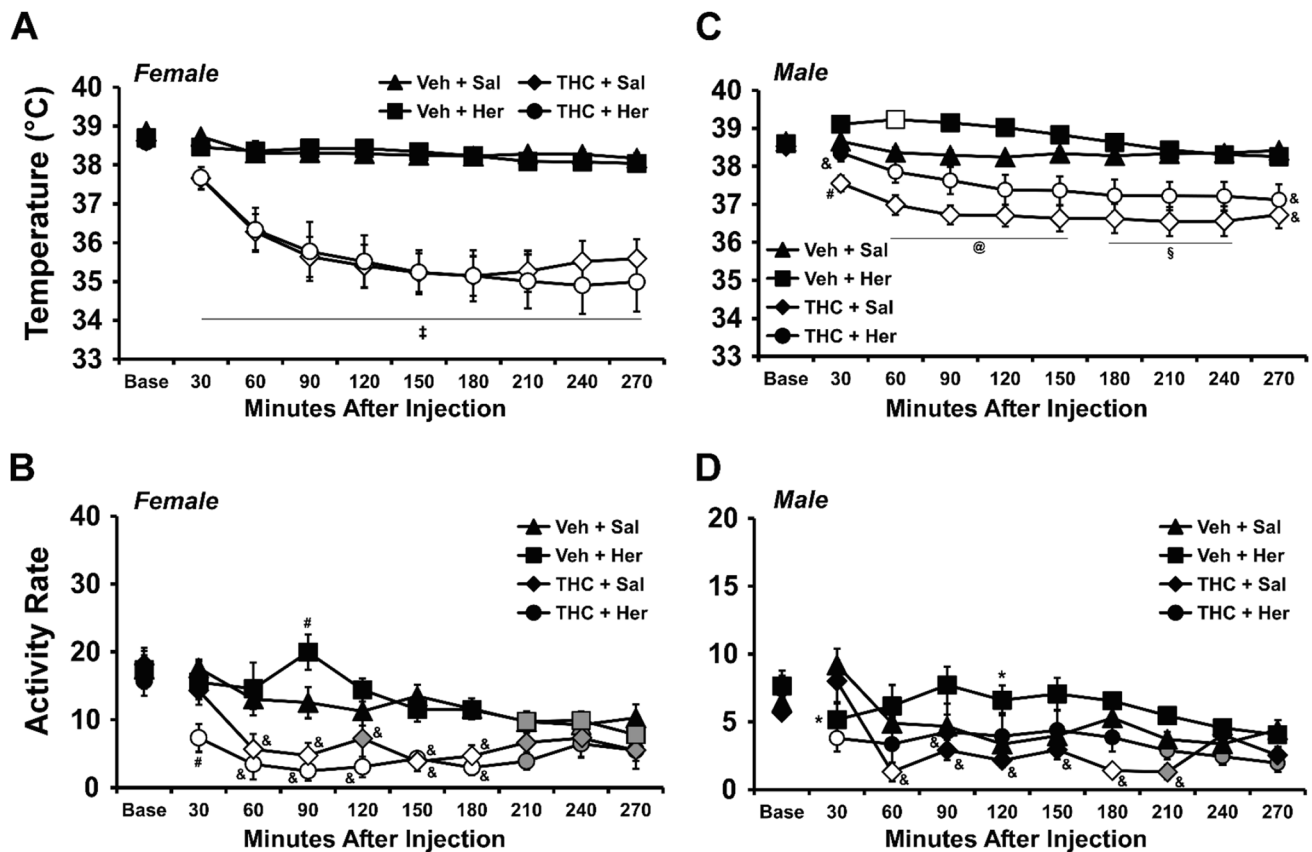
Fig. 3 Mean ($\pm$ SEM) body temperature (**A**, **C**) and activity rate (**B**, **D**) for female ($N = 7$; **A**, **B**) and male ($N = 6$; **C**, **D**) Sprague–Dawley rats after injection of THC (10.0 mg/kg, i.p.), heroin (0.5 mg/kg, s.c.), the 1:1:18 *veh*icle or *sal*ine in the indicated combinations. N.b. there is a twofold difference in the scale of the activity in **B** and **D**, to accommodate the sex difference in activity rate. Open symbols indicate a significant difference from both the vehicle at a given time-point and the within-treatment baseline, while shaded symbols indicate a significant difference from the baseline, within treatment condition, only. A significant difference of all treatment conditions from each other at a given time post-injection is indicated with a commercial At, a difference between all active drug conditions with a section sign, and a difference of each of the THC conditions from both of the vehicle conditions with a double dagger. A significant difference from the PG inhalation condition (but not the baseline) is indicated by an asterisk, a difference from the heroin condition with an ampersand, and from all other conditions with a number sign

the vehicle + saline injection condition after THC + saline (60–90, 150–180 min) or THC + heroin (60–180 min), reduced compared with all other conditions 30 min after injection of THC + heroin and increased compared with all other conditions 90 min after injection of vehicle + heroin significantly lower activity compared with heroin alone was also observed in the THC + heroin condition (30–180 min after the start of inhalation) as well as the THC condition (60–180 min).

The body temperature of the male rats was significantly elevated by heroin and decreased by THC (Fig. 3C). The ANOVA confirmed a significant effect of time after injection (F (9, 54) = 26.79; $P < 0.0001$), of drug treatment condition (F (3, 18) = 15.12; $P < 0.0001$) and of the interaction of time with drug condition (F (27, 162) = 6.26; $P < 0.0001$) on body temperature. The post hoc test confirmed that temperature was changed relative to the

baseline, and also relative to the vehicle/saline injection condition, after the injection of THC (30–270 min post-injection), heroin (60 min), or the THC + heroin combination (60–270 min). Body temperature was also significantly lower compared with the heroin condition after the injection of either THC (30–270) or the THC + heroin combination (30–270 min).

The activity of the male rats (Fig. 3D) was increased by heroin injection and decreased by the THC injection as confirmed by a significant effect of time after injection (F (9, 54) = 6.27; $P < 0.0001$) of drug treatment condition (F (3, 18) = 9.20; $P = 0.001$) and of the interaction of time with drug condition (F (27, 162) = 2.34; $P < 0.001$) on activity in the ANOVA. The post hoc test confirmed that activity rates were reduced compared with the baseline and the vehicle + saline injection condition after THC + saline (60, 180 min after injection) or THC + heroin (30 min), and

increased relative to the vehicle + saline injection condition after vehicle + heroin (120 min after injection).

Experiment 4 (nociception in male and female Sprague–Dawley rats)

The ANOVA confirmed that sex ($F (1, 12) = 10.10$; $P < 0.01$) and vapor condition (F (4, 48) = 27.94; $P < 0.0001$) significantly altered the tail withdrawal latency (Fig. 4). The post hoc test failed to confirm any specific sex differences within any of the vapor conditions. Within the female or male groups, a significant difference from the PG condition was observed after the inhalation of heroin or the combination of heroin and THC. Tail withdrawal was likewise significantly slower after the inhalation of the combination relative to the THC inhalation in the female rats and relative to the THC or Heroin conditions in the male rats.

## Experiment 5 (naloxone antagonism in male Sprague–Dawley rats)

Temperature and activity

Pre-treatment with naloxone attenuated the temperature and locomotor responses to heroin inhalation (Fig. 5). One individual exhibited an anomalous temperature response to each heroin condition and was omitted from the final analyses; thus, $N = 6$ for this study. As in experiment 2, after inhaling the vehicle vapor, the body temperature of rats declined slightly across the first 3 h, and the prior injection of naloxone had no impact.
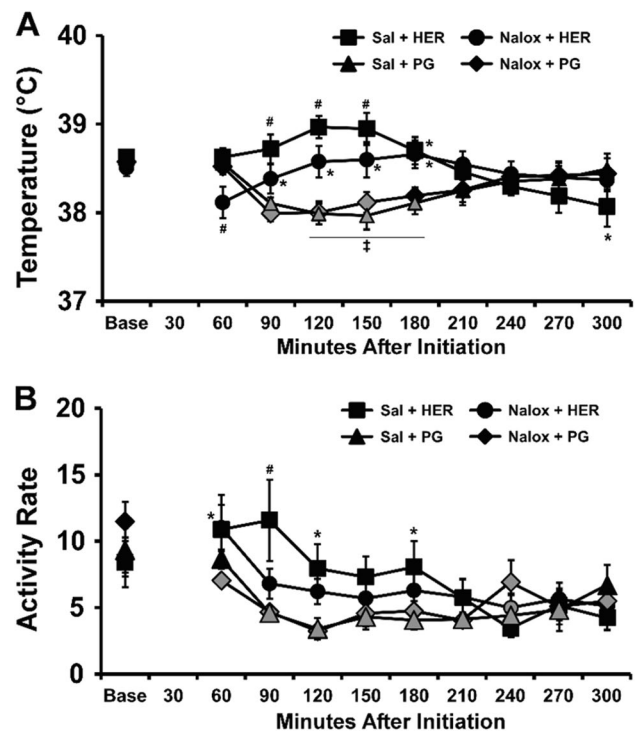
Fig. 5 Mean ($N = 7$; $\pm$ SEM) temperature (**A**) and activity (**B**) for male Sprague–Dawley rats after injection with either *sal*ine or *nalox*one (1.0 mg/kg, i.p.) and propylene glycol (PG) or heroin (HER; 100 mg/mL) vapor exposure for 30 min. Shaded symbols indicate a significant difference from the *Base*line, within the treatment condition. A significant difference of each of the heroin vapor conditions from each of the PG conditions is indicated with a double dagger. A significant difference from the PG inhalation condition (within pretreatment) is indicated by an asterisk, and a difference compared with all other conditions with a number sign

Fig. 4 Mean ($\pm$ SEM) tail withdrawal latency for male and female ($N = 7$ per group) rats after vapor exposure. A significant difference from PG condition is indicated with an asterisk and a difference from the THC + heroin condition with a number sign. PG, propylene glycol. Heroin (50 mg/mL); THC (50 mg/mL)
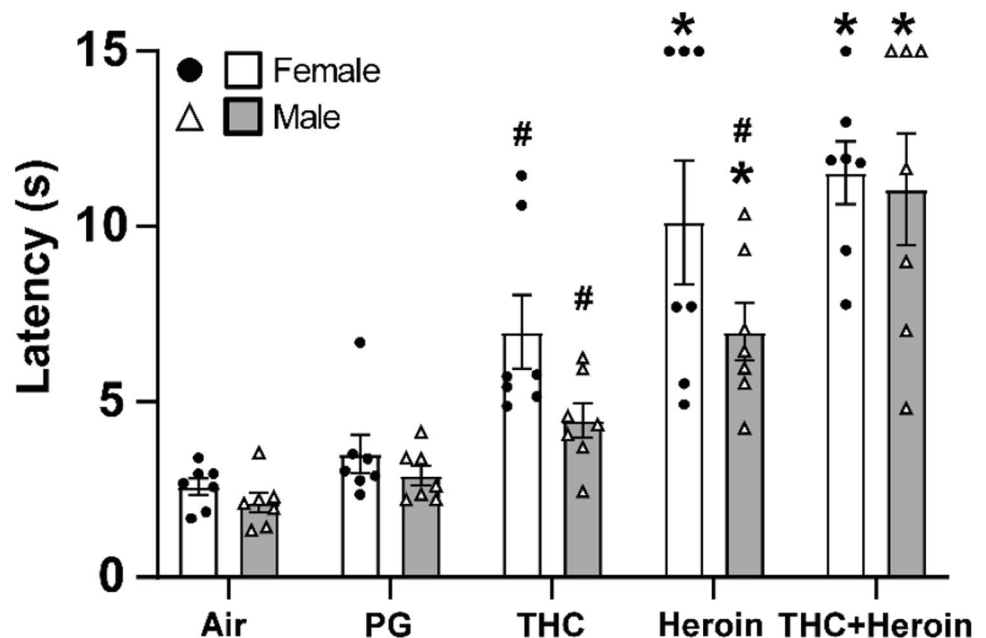
The inhalation of heroin (100 mg/mL) for 30 min resulted in higher body temperature in the first 2 h after the start of inhalation, compared with the PG inhalation conditions (Fig. 5A); again, this was similar to the outcome of heroin inhalation in experiment 2. Naloxone pre-treatment attenuated the temperature response to heroin. The three-way ANOVA confirmed a significant effect of the interaction of vapor condition with Time after the start of inhalation ($F_{(9, 45)} = 15.45$; $P < 0.0001$) of the interaction of pre-treatment condition with time after the start of inhalation ($F_{(9, 45)} = 2.90$; $P < 0.01$) and the interaction of all three factors ($F_{(9, 45)} = 3.57$; $P < 0.005$) on body temperature. The post hoc test confirmed first that heroin inhalation resulted in increased body temperature relative to PG inhalation 90–180 min after initiation. In addition, while naloxone had no impact on temperature when administered prior to PG inhalation, it attenuated the increase in body temperature observed 90–150 min after the start of Heroin inhalation. Activity was also impacted by heroin inhalation and the three-way ANOVA confirmed a significant effect of Time after the start of inhalation ($F_{(9, 45)} = 8.60$; $P < 0.0001$) of vapor condition ($F_{(1, 5)} = 7.17$; $P < 0.05$) of the interaction of vapor condition with time after the start of inhalation ($F_{(9, 45)} = 5.09$; $P < 0.0001$) on activity. The post hoc test confirmed that naloxone pre-treatment caused significant attenuation of heroin-stimulated activity 90 min after the start of inhalation.

Anti-nociception

The tail withdrawal latency was increased by heroin vapor inhalation and this effect was attenuated by the pre-inhalation injection of naloxone. The ANOVA confirmed significant effects of vapor condition ($F_{(1, 6)} = 20.18$; $P < 0.005$) and of the interaction of pre-treatment and vapor conditions ($F_{(1, 6)} = 6.69$; $P < 0.05$), but not of pre-treatment alone ($F_{(1, 6)} = 5.94$; $P = 0.051$)), on tail withdrawal latency (Fig. 6). The post hoc test confirmed that this was attributable to tail-withdrawal latency being longer in the saline-heroin vapor condition compared with the other three treatment conditions.

Experiment 6 (nociception in male and female Wistar rats)

For analysis, each of the three tail-withdrawal assays on each of four treatment sequence days was considered as a unique observation condition (Fig. 7). This resulted in four pre-treatment observations, two after the 1:1:18 vehicle injection, two after THC (10 mg/kg, i.p.) injection, and one and the end of each of the vehicle + saline, vehicle + heroin, THC + saline, and THC + heroin sequences. The initial three-way ANOVA confirmed significant effects of sex ($F_{(1, 216)} = 13.75$; $P < 0.0005$), of the observation condition ($F_{(11, 216)} = 39.87$; $P < 0.0001$) and an interaction of sex with adolescent treatment ($F_{(1, 216)} = 4.29$; $P < 0.05$) on tail- withdrawal latency in the Wistar rats. The

Fig. 6 Mean (± SEM) tail withdrawal latency for male ($N = 7$) Sprague–Dawley rats after 30 min of heroin vapor exposure, following injection of saline or naloxone (1.0 mg/kg, i.p.). A significant difference across inhalation conditions is indicated with an ampersand, a significant difference from PG inhalation, within pre-treatment condition, is indicated with an asterisk, and a difference from the saline pre-treatment within inhalation condition with a number sign. PG, propylene glycol. Heroin (100 mg/mL)
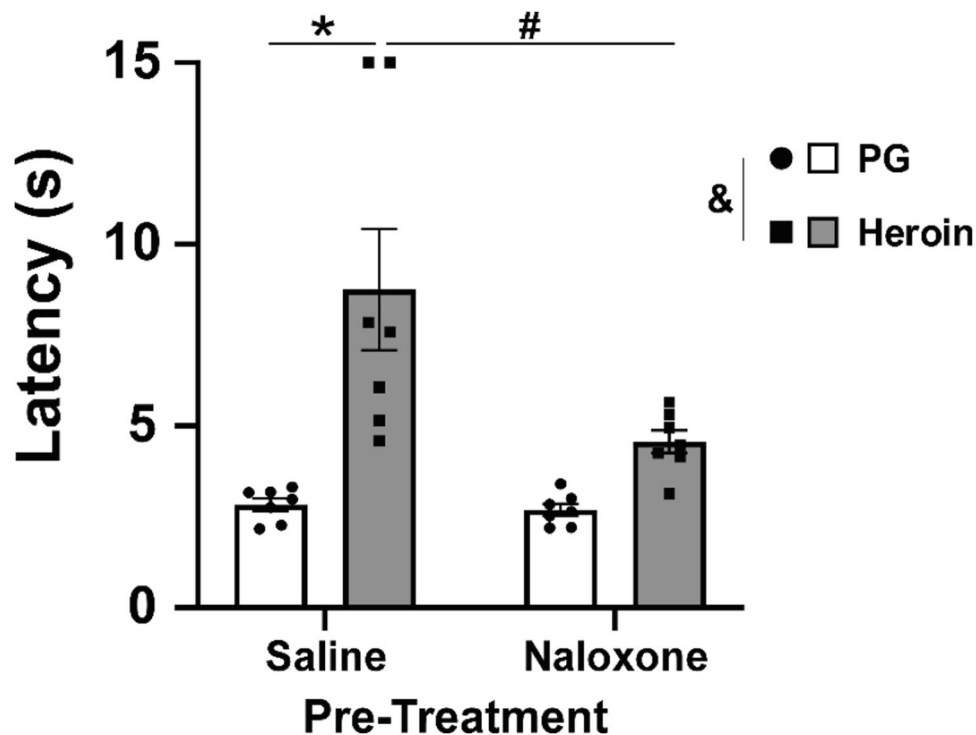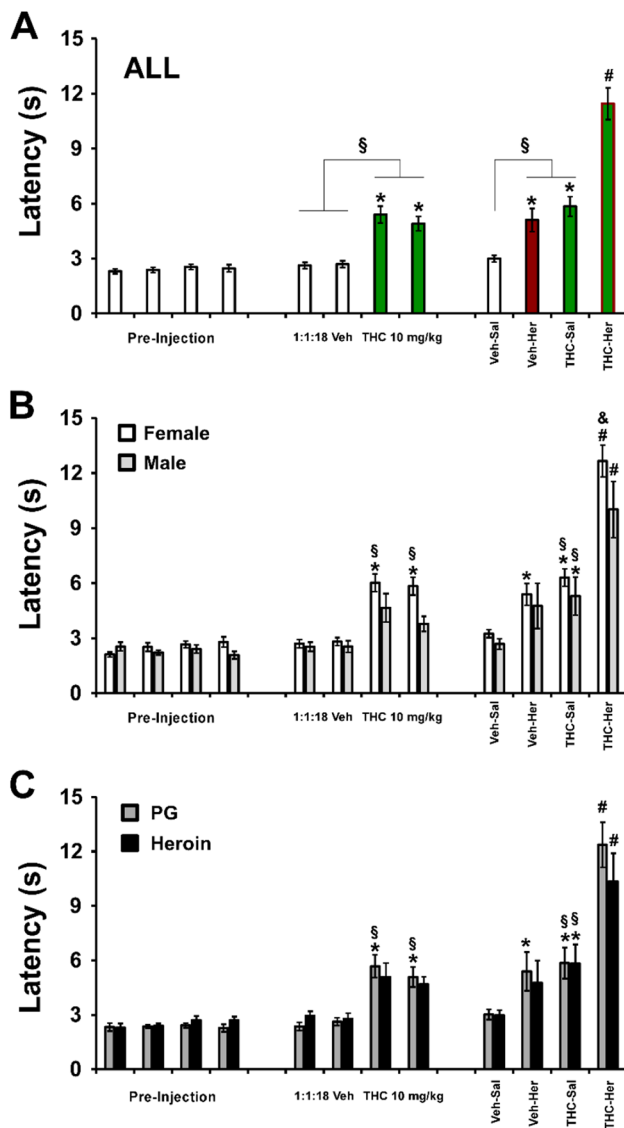
Fig. 7 A Mean (±SEM) tail withdrawal latency for all rats (*N* = 22) at three timepoints within each of four treatment conditions/sessions. The first injection of the session was either THC (10 mg/kg, i.p.) or the cannabinoid vehicle and the second injection was either saline or heroin (0.5 mg/kg, s.c.) for four total treatment conditions. **B** Withdrawal latency for female (*N* = 12) and male (*N* = 10) subgroups. **C** Withdrawal latency for subgroups of rats treated with repeated PG (*N* = 12) or heroin (*N* = 10) vapor as adolescents. Within groups, a significant difference from the respective pre-injection baseline is indicated with an asterisk, a difference from the respective vehicle or saline condition with a section sign, a difference from all other treatment conditions with a number sign. A difference between groups is indicated with an ampersand

first follow-up two-way ANOVA collapsed across adolescent treatment (Fig. 7B) confirmed a significant effect of observation condition (F (11, 220) = 49.85; $P < 0.0001$) and of sex (F (1, 20) = 4.66; $P < 0.05$). The post hoc test of the marginal means for treatment condition confirmed that tail-withdrawal latency was higher after each of the THC

injections compared with all of the pre-treatment latencies and with the two latencies after the 1:1:18 vehicle injections. It was further confirmed that latencies were higher after the Heroin injection in the Vehicle condition and after the saline injection in the THC condition compared with the respective pre-injection baselines and with the latency in the vehicle + saline condition. Finally, tail-withdrawal latencies after the heroin injection in the THC condition were significantly higher than in any other condition. Within sex, these patterns were similarly confirmed for the female group. Within the male subgroup, withdrawal latencies were higher after the THC + heroin condition compared with all other treatment conditions and in the THC + saline condition compared with the respective pre-treatment baseline and the vehicle + saline condition. The only sex difference confirmed within a specific treatment condition was after injection of both THC and heroin. Collapsed across sex, there was no significant effect of adolescent exposure group, an effect of observation condition (F (11, 220) = 48.81; $P < 0.0001$), and the post hoc test recapitulated the overall outcome (Fig. 7C).

Experiment 7 (nociception in experimentally naive female Wistar rats)

The injection of heroin (0.1–1.0 mg/kg, s.c.) by itself produced dose-dependent increases in tail-withdrawal in the female Wistar rats (*N* = 10), as shown in Fig. 8A. The ANOVA confirmed a significant effect of dose (F (2.031, 18.28) = 31.89; $P < 0.0001$), and the post hoc test confirmed that withdrawal latency was longer after 0.56 mg/kg, compared with the vehicle injection, and longer after 1.0 mg/kg compared with all other conditions.

Using the threshold doses of heroin, it was then shown that interactive effects with THC (5 mg/kg, i.p.) were only observed at the 0.56 mg/kg dose of heroin (Fig. 8B, C). Analysis included a factor for the within-session time of observation as well as the final heroin dose condition. The two-way ANOVA within the *vehicle pre-treatment condition* first confirmed a significant effect of the within-session assessment time (F (2, 18) = 24.42; $P < 0.0001$), but not of heroin dose condition or the interaction of the factors, on withdrawal latency (Fig. 8B). The post hoc confirmed that this was attributable to a significant increase compared with the pre-treatment assessment after the vehicle injection for the heroin 0.1 mg/kg treatment, and a difference of the post-heroin 0.56 mg/kg latency compared with the pretreatment and post-vehicle assessment on that same session. In contrast, the two-way ANOVA within the *THC 5.0 mg/kg, i.p., pre-treatment condition* confirmed an effect of the within-session assessment time (F (2, 18) = 77.73; $P < 0.0001$), of heroin dose condition (F (3, 27) = 10.96; $P < 0.0001$) and of the interaction of the factors (F (6, 54) = 5.14; $P < 0.0005$) on tail withdrawal latency (Fig. 8C). The post hoc test first
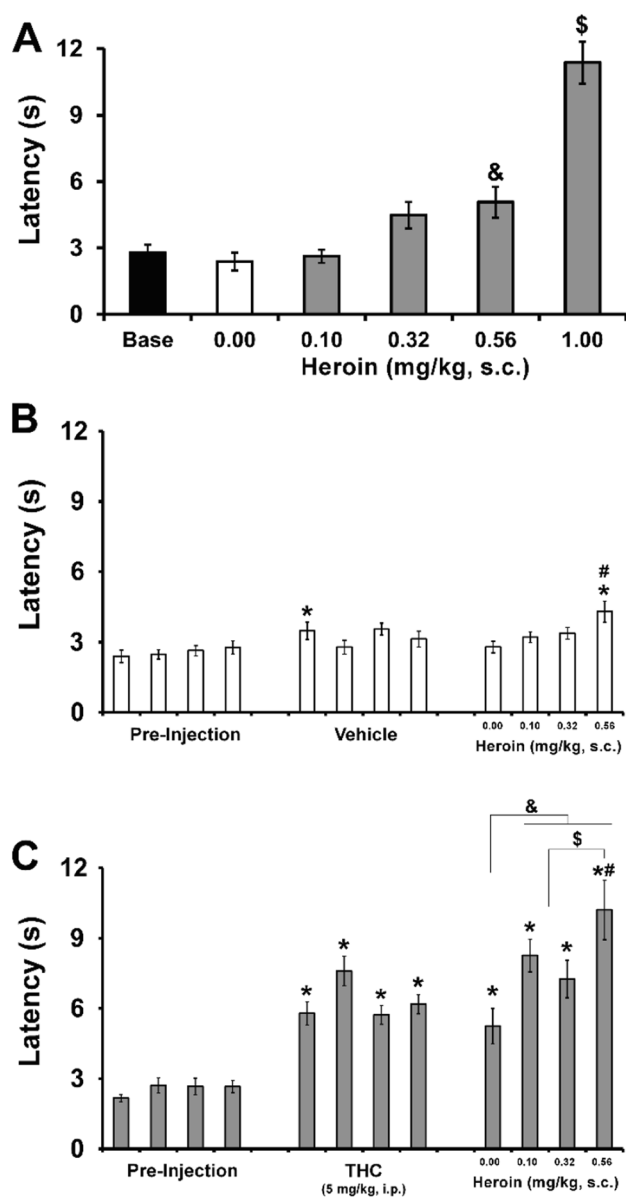
Fig. 8 A Mean (± SEM) tail-withdrawal latency in a group (N = 10) of female Wistar rats evaluated after no treatment (*base*line) or after injection with saline or heroin (0.1–0.56 mg/kg, s.c.). Mean (± SEM) tail-withdrawal latency in a group (N = 10) of female Wistar rats evaluated before injection, then after either **B** the 1:1:18 vehicle or **C** THC 5 mg/kg, i.p., then after heroin. A significant difference from the saline condition is indicated by an ampersand and a difference from all other conditions with a dollar sign. Within-session, a significant difference from the pre-injection value is indicated by an asterisk and a difference from the vehicle/THC injection with a number sign. Across sessions, a significant difference from the saline (0.0) condition is indicated with an ampersand and from the 0.56 dose with a dollar sign

confirmed that the effect of heroin dose (on the final assessment of the session) was attributable to a significant increase of latency in all three active dose conditions compared with saline, and an elevation after heroin 0.56 mg/kg compared

with the 0.1 or 0.32 mg/kg conditions. The post hoc test further confirmed that the THC injections increased tail-withdrawal latency compared with the pre-treatment assessment within all four sessions. Heroin only significantly increased latency beyond the effect of the THC injection in the 0.56 mg/kg condition.

Most critically, a two-way ANOVA analyzing the withdrawal latency after the last observation of all sessions (i.e., after heroin injection in both THC and vehicle pre-treatment conditions) confirmed a significant effect of the heroin dose ($F (3, 27) = 11.46$; $P < 0.0001$), of the vehicle/THC pretreatment ($F (1, 9) = 46.94$; $P < 0.0001$) and of the interaction of factors ($F (3, 27) = 4.30$; $P < 0.05$) on tail-withdrawal latency. The post hoc test further confirmed a significant difference between saline injection and all three heroin doses, and between 0.32 and 0.56 mg/kg, within the THC pre-treatment condition. There were no effects of heroin dose in the vehicle pretreatment conditions confirmed in this analysis.

Discussion

This study first confirms that THC enhances the anti-nociceptive effect of heroin when the drugs were administered by vapor inhalation via an electronic drug delivery system (EDDS) (Fig. 4), in a manner similar to the effects observed when the drugs were delivered by parenteral injection (Figs. 7 and 8). This outcome is consistent with a prior demonstration that THC enhances the effects of another opioid, oxycodone, on anti-nociception when drugs are delivered either by inhalation or injection (Nguyen et al. 2019). Thus, the routes of administration, and the doses used in this study, are those which are effective at demonstrating the "opioid-sparing" effects of THC when it comes to one desired medical benefit of both opioids and THC, i.e., analgesia. In contrast, the effects of each drug on thermoregulation and locomotor activity when co-administered appear to be independent. That is, when doses of heroin that increase body temperature are combined with doses of THC that decrease body temperature, the net effect is an intermediate response, indistinguishable from the response to vehicle in some cases. Interpretation of the effects of co-administration on activity is complex because the effects of higher doses of heroin are biphasic with time after dosing. Still, at a time post-administration when *hyper*locomotive effects of heroin are observed, the combination with *hypo*locomotor doses of THC results in an intermediate effect on activity.

There is a biphasic dose-dependent effect of heroin on body temperature with lower exposures producing small, but reliable, increases in temperature and higher doses/exposures reducing body temperature, as with the first experiment (Fig. 1) and in female rats in a prior report (Gutierrez et al. 2021). Here, we selected parameters of exposure to heroin

anticipated to increase body temperature in both male and female rats and a parameter of THC exposure anticipated to lower body temperature. The results show (Figs. 1B and 2A, B) that the co-administration produces an intermediate phenotype, consistent with independent effects. This was the case in two cohorts of male rats as well as in the female rats, illustrating the robustness of the observation. There was a slight disconnect in that inhalation of vapor from heroin 50 mg/mL for 30 min was the higher exposure in the first group and constituted a lower (phenotypic) exposure in the second group of males. It is likely that the slight change in methods across studies produced the difference, i.e., we used multiple inhalation chamber setups. This further underlines the necessity for validation studies to hone exposure conditions when using this method, even when the same canister/atomizer and power supply ("mod") configurations are used.

We have previously shown that heroin vapor exposure (100 mg/mL, 30 min) produces anti-nociceptive effects in male and female Wistar rats (Gutierrez et al. 2020b) and that is herein extended to Sprague–Dawley rats. We also show here that the competitive, non-selective opioid receptor antagonist naloxone attenuates the anti-nociceptive effects of inhaled heroin (Fig. 6). It also blunted the thermoregulatory and locomotor stimulant responses to heroin inhalation (Fig. 5). This is a novel observation since we did not include this in our prior study (Gutierrez et al. 2021) which was the first to show the efficacy of EDDS vapor delivery of heroin. While the effect of naloxone is perhaps expected, we have reported an unanticipated lack of effect of CB_1 antagonist/inverse agonist pre-treatment on THC vapor–induced hypothermia (Nguyen et al. 2020), so it was important to confirm opioid antagonist efficacy in this model. The failure to achieve a statistically reliable additive anti-nociceptive effect of THC + heroin inhalation in the female group is likely due to a slightly more robust response to the heroin inhalation condition, compared with the males. We then went on to expand this experiment to a broader range of conditions in female Wistar rats, using drug injection to afford tighter dose control (Fig. 8). These studies demonstrated that a 0.56 mg/kg, s.c., dose of heroin combined with a 5.0 mg/kg, i.p. dose of THC appears to approximate the threshold for observable interactions. After demonstrating that the 0.1–0.32 mg/kg, s.c., doses of heroin produce minimal effect administered alone, we then went on to show in the complex design that only 0.56 mg/kg produced a significant increase in nociception beyond that induced by 5.0 mg/kg, i.p., THC. The 5.0 and 10.0 mg/kg THC doses appear to produce approximately the same magnitude of effect in the tail withdrawal assay at 52 °C as illustrated here (Figs. 7 and 8) and in prior work (Nguyen et al. 2016b, 2019), suggesting an asymptote in the dose–response curve for this drug when administered alone. It is therefore intriguing that the addition of 0.5 mg/kg heroin to 10 mg/kg THC had a greater additive anti-nociceptive effect than the addition of 0.56 mg/kg heroin to 5 mg/kg THC. This hints at a more than the additive interactive effect that would need to be explored with additional experimental designs to confirm. As one minor caveat, although the two female groups were well age-matched, the second group was experimentally naïve and the first group had received prior drug exposure. Overall, these results confirm the so-called opioid-sparing effects of THC in the context of thermal analgesia. The translational potential is that by taking lower doses of each drug the consequences of higher doses might be partially avoided. This may be important for slowing the development of tolerance to the therapeutic benefits of either drug independently and thereby slow the need for increasing doses.

In a more general sense, this study further confirms the utility of the EDDS approach for the investigation of poly-substance use. Patterns of polydrug use in humans via simultaneous vaping are already being described in the epidemiological literature (Dunbar et al. 2020), which tends to lag real-world practices by months to years; thus, it is a critical advance to be able to study such practices in a controlled laboratory model. As outlined in the Introduction, EDDS models for the inhalation delivery of a range of drugs in laboratory rodents are being rapidly developed and reported. This technique for laboratory rodent research is flexible in terms of drug substances and doses and is therefore capable of supporting poly-drug investigations. We have previously used this model to explore interactive effects of nicotine with THC (Javadi-Paydar et al. 2019b) and of nicotine with cannabidiol (Javadi-Paydar et al. 2019a), as well as the effects of combined inhalation of these two cannabinoids (Javadi-Paydar et al. 2018). As a minor caveat, we did not examine both sexes in every experiment. In the studies that did examine the sexes in parallel, the effects appeared to be qualitatively similar, albeit there may be minor differences in dose–effect curves. Thus, although there is support for the conclusion that studies conducted in one sex would generalize, a firm conclusion would await future verification in direct sex comparisons.

In conclusion, THC can reduce the dose of heroin necessary for a given analgesic effect. However, the effects of combined THC and heroin on activity and thermoregulation show, critically, that the inference that THC *universally* enhances the effects of heroin, or vice versa, is not supported. When the effects of each drug in isolation are in the same direction, due to either dose or the in vivo endpoint, they can appear to have interactive effects. However, when the effects are in the opposite direction, such as with body temperature and activity at specific doses, then the combination produces an intermediate phenotypic outcome. The anti-nociception data do not suggest any super-additive interactive effects of THC with heroin. While a super-additive interaction might appear beneficial for medical applications

the limitation would be that any tolerance to one or the other drug that appears would also have interactive, rather than merely subtractive effects. Additional study would be required to determine whether combinations of cannabinoid and opioid drugs used chronically result in less rapid tolerance compared with equipotent therapeutic regimens of each drug taken alone. Relatedly, it remains to be determined if the ability to more closely titrate dose to effect can be obtained with the inhaled route of administration of THC or an opioid.

Declarations

Conflict of interest The authors declare no competing interests.

Disclaimer The NIH/NIDA and the TRDRP had no role in study design, collection, analysis, and interpretation of data, in the writing of the report, or in the decision to submit the paper for publication.

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