Self-administration of heroin by vapor inhalation in female Wistar rats

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Abstract

Over the last two decades the United States has experienced a significant increase in the abuse of opioid drugs, resulting in record numbers of opioid-related overdoses and deaths. Starting around 2002, there has been an increase in non-medical use of prescription opioids, followed by increased heroin and then fentanyl use. Inhalation has been a common route of administration for opioid drugs, with a documented history spanning back to Mediterranean antiquity and up through modern use with e-cigarette devices, but preclinical reports with inhalation as the model route of administration remain relatively few. This study was conducted to determine the efficacy of e-cigarette vapor inhalation of heroin in rats. Non-contingent exposure to heroin vapor produced slowed tail-withdrawal in male and female rats, demonstrating anti-nociceptive efficacy. Female Wistar rats trained to self-administer heroin vapor exhibited individual preference differences. The most-preferring half of the distribution administered more vapor reinforcers when the concentration of heroin was reduced in the vapor vehicle and when pre-treated with the opioid receptor antagonist naloxone. The anti-nociceptive effect of heroin self-administered by vapor was identical in magnitude to that produced by intravenous self-administration. Finally, anxiety-like behavior increased 24-48 h after last heroin vapor access, consistent with withdrawal signs seen with intravenous self-administration. In sum, these studies show that pharmacological effects of heroin are produced in rats by vapor inhalation using e-cigarette technology. Importantly, self-administration models by this route can be deployed to determine health effects of inhaled heroin.

1. Introduction

Over the past two decades the United States has experienced a significant increase in the problematic use of opioid drugs. The trend started with prescription opioids circa 2002, followed by an increase in the use of heroin starting around 2012, and eventually added illicit fentanyl starting in 2015; this all resulted in record numbers of opioid-related overdoses and deaths (Rudd et al., 2016b; Rudd et al., 2016a; Hedegaard et al., 2018; Scholl et al., 2018). Progression in use of prescription drugs into illicit street opioids often results in progression to more direct routes of administration, such as intravenous injection or inhalation of the volatilized drug (Mars et al., 2014; Woodcock et al., 2015). While the effects of injected opioids in humans and animals have been studied extensively for decades, study of the effects of inhaled drugs in animals have been much less common partly due to the lack of effective means of drug delivery and, consequently, varying methods of volatilization and experimental parameters. The recent popularity of electronic nicotine delivery devices (ENDS), and their adaptation for research purposes, has greatly facilitated the study of the effects that drugs produce via inhalation.

Inhalation has been a common route of administration for opioid drugs, with a documented history spanning back to Mediterranean antiquity, as reviewed (Kritikos, 1960). Some of the earliest modern Western opioid misuse crises occurred in the 19th century during an interval of Chinese diaspora and involved the inhalation of opium vapor (Kane, 1881). Inhalation presently continues to be a common route of administration among individuals that abuse opioids (Alambyan et al., 2018) and some newer users will first choose inhalation as a means of administering opioids because of the perceived safety compared with injection (Stover and Schaffer, 2014). Although some degree of safety is achieved, such as in the prevention of bloodborne pathogen transmission, inhalation and injection both similarly facilitate progression into dependence (Barrio et al., 2001). Further, the subjective and physiological effects of inhaled heroin are similar to those of injected heroin, but plasma levels may decline more quickly (Jenkins et al., 1994); this might support more frequent re-dosing. Inhalation of opioids is typically achieved by heating the drug over a surface, often tin foil, and inhaling the resulting vapors (Jenkins et al., 1994; Strang et al., 1997). Recently, however, there have been reports of ENDS usage (vaping) for administering illicit substances, including opioid drugs (Kenne et al., 2017; Blundell et al., 2018; Breitbarth et al., 2018). The current popularity and acceptability of vaping, the covert and easily concealed designs of newer vaping devices, and the myriad

flavorant options, which can potentially mask public use, make this latter method especially concerning. Because non-medical use of opioids by inhalation remains prevalent, further determination of any route-specific effects warrant attention.

Despite numerous epidemiological and clinical studies on human usage of inhaled opioid drugs, preclinical reports which use inhalation as the route of administration remain relatively scarce. Antinociceptive effects can be produced, as measured by the tail withdrawal test, following passive exposure to vaporized morphine, heroin, and fentanyl in mice (Lichtman et al., 1996) and oxycodone in rats (Nguyen et al., 2019b). Additionally, rhesus monkeys will self-administer heroin by inhalation and will exhibit behaviors such as drug-seeking upon increase of the reinforcement contingency and dose-dependent suppression of drug-taking behavior when pre-treated with naloxone (Mattox and Carroll, 1996; Mattox et al., 1997) that are characteristic of heroin intravenous self-administration. Adult rats will self-administer the synthetic opioid sufentanil in aerosol mist form delivered through a nebulizer (Jaffe et al., 1989) and through the use of a vapor delivery system based on e-cigarette technology (Vendruscolo et al., 2018). One provocative new study may even constitute evidence for the self-administration of a cannabis extract in rats via vapor inhalation (Freels et al., 2020). The opioid self-administration studies found behavioral effects comparable to those seen in rodent intravenous self-administration studies, such as concentration-dependent responding for drug, and the Vendruscolo et al. (2018) study additionally demonstrated escalation after extended drug access and drug-seeking behavior in naloxone pre-treated rats (Jaffe et al., 1989; Vendruscolo et al., 2018). Together, these studies demonstrate the viability of self-administration models of inhaled drugs and illustrate some of the behavioral similarities between the effects produced by inhalation and intravenous injection of opioid drugs. However, the limited number of available reports on the behavioral effects of opioid inhalation makes any conclusions on behavioral similarities or differences due to route of administration difficult to make. Further work is therefore required to determine the specific reinforcing properties of individual opioid drugs delivered by ENDS and other devices, especially under differing conditions of volatilization.

The present study was therefore designed to assess the effects of heroin delivered by ENDS vapor inhalation on measures of reinforcement, withdrawal-like behavior, and nociception. The approach adapted a technique we have shown delivers behaviorally active, non-contingent doses of drugs as varied as methamphetamine and MDPV (Nguyen et al., 2016a; Nguyen et al., 2017), Δ^9-tetrahydrocannabinol and cannabidiol (Nguyen et al., 2016b; Javadi-Paydar et al., 2018; Javadi-Paydar et al., 2019a), oxycodone and sufentanil,

(Vendruscolo et al., 2018; Nguyen et al., 2019b) and nicotine (Javadi-Paydar et al., 2019b). To determine behavioral efficacy of inhaled heroin, we first assessed anti-nociception by measuring tail withdrawal latency from a hot water bath after non-contingent opioid vapor exposure and compared this with the effects of subcutaneous opioid injection. Next, groups of adult female Wistar rats were trained to lever-press for delivery of in-chamber heroin vapor in 30 second epochs. Female rats were selected for this study first because the prior inhalation studies used male rats (Jaffe et al., 1989; Vendruscolo et al., 2018) and it is important to determine if effects generalize to female subjects (Cicero et al., 2003; Clayton and Collins, 2014; Shansky and Woolley, 2016; Shansky, 2018; Nguyen et al., 2019a). Furthermore, there is some indication female rats more readily self-administer more morphine, heroin, oxycodone, or fentanyl compared with male rats (Klein et al., 1997; Cicero et al., 2003; Nguyen et al., 2020), thus female rats were also anticipated to speed model development. Following acquisition of vapor self-administration, drug concentrations in vapor solution were varied and behavioral response was assessed. Drug-seeking behavior was also examined after the administration of the antagonist naloxone to provide converging evidence on the pharmacological specificity of the behavior. Tail withdrawal measurements were also performed on the self-administration rats before and after a self-administration session to verify that an active dose was self-administered. Finally, anxiety-like behavior was probed immediately, 24-, and 48-hours post-session using an elevated plus maze approach.

2. Methods

2.1 Animals

Adult female (N=8) and male (N=8) Wistar rats (Charles Rivers Laboratories) were used for non-contingent vapor and injection studies. Adult female (N=24) and male (N=8) Wistar rats (Charles Rivers Laboratories) were used for self-administration studies. Rats were acclimated to the facility's vivarium upon arrival for two weeks prior to initiating studies. The vivarium was kept on a 12:12 hour light-dark cycle and behavior studies were conducted during the rats' dark period. Food and water were provided *ad libitum* in the home cage. Animal body weights were recorded weekly. Two experimental cohorts of female rats were used for vapor self-administration experiments (N=16 total). The first subgroup (N=8), designated Cohort 1 (C1), underwent acquisition training, nociception testing, and concentration substitution experiments (further described below). Animals in Cohort 2 (C2) were tested as described for C1 but were

returned to self-administration prior to being challenged with naloxone and assessed in an elevated plus maze (EPM) for anxiety-like behavior after spontaneous withdrawal. Experimental procedures were conducted in accordance with protocols approved by the IACUC of The Scripps Research Institute and consistent with recommendations in the NIH Guide (Garber et al., 2011).

2.2 Drugs

Heroin (diamorphine HCl) was administered by s.c. injection and by vapor inhalation. Methadone was administered by s.c. injection and naloxone by i.p. injection. Inhalation/vapor doses are varied, and are therefore described, by altering the concentration in the propylene glycol (PG) vehicle, the puff schedule and/or the duration of inhalation sessions in this approach (Nguyen et al., 2016a; Nguyen et al., 2016b; Javadi-Paydar et al., 2019b). The heroin was dissolved in the PG to achieve target concentrations and then loaded in the e-cigarette tanks in a volume of ~ 0.5 ml per session. Fresh solutions were used for each session. Naloxone, methadone and heroin were dissolved in saline (0.9% NaCl), for injection. The heroin was provided by the U.S. National Institute on Drug Abuse and the PG, methadone and naloxone were obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA).

2.3 Electronic vapor drug delivery

Passive opioid vapor exposure sessions prior to the initial nociception experiments were conducted in modified Allentown rat cages (259mm X 234mm X 209mm) with the use of Model SSV-1 e-vape controllers (La Jolla Alcohol Research, Inc, La Jolla, CA) to trigger Protank 3 Atomizers (Kanger Tech, Shenzhen Kanger Technology Co. LTD; Fuyong Town, Shenzhen, China) pre-filled with drug solution. A computerized controller (Control Cube 1; La Jolla Alcohol Research, Inc, La Jolla, CA, USA) was used to automatically trigger ten-second vapor puffs at five-minute intervals. Vacuum exhausts attached to the chambers pulled ambient air through intake valves at ~2 L per minute, starting 30 seconds before each scheduled vapor delivery. Vacuum exhausts were closed immediately following each vapor delivery. Duration for passive vapor exposure sessions was 30 minutes.

For self-administration rats, vapor was delivered into sealed vapor exposure chambers (152 mm W X 178 mm H X 330 mm L; La Jolla Alcohol Research, Inc, La Jolla, CA, USA)

through the use of e-vape controllers (Model SSV-3; 58 watts; 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 ohm coils. Vapor exposure chambers were attached to vacuum exhausts which continuously pulled ambient air through intake valves at ~1.5-2 L per minute. Vapor deliveries were 1 second in duration for self-administration sessions, and drug vapor from each delivery was mixed with flowing air entering the chamber. These parameters resulted in a vapor dwell time of approximately 30 seconds, in those cases where no further vapor deliveries had been obtained.

2.4 Behavioral Responses to Vapor Inhalation of Heroin

2.4.1 Nociception assays

Tail withdrawal assays were performed using a Branson Brainsonic CPXH Ultrasonic Bath (Danbury, CT) filled with water and set and maintained at a temperature of 52ºC. The bath was stirred, and temperature was verified using a second thermometer prior to each measurement. A stopwatch was used to measure the latency to tail withdrawal, and a 15-second maximum time limit to withdraw was imposed for each session.

Since efficacy of this system for heroin delivery has not been reported, initial studies were conducted to verify vapor exposure parameters that would induce significant anti-nociception, to compare this to an injected dose commonly reported in precedent literature and to determine if effects generalized to another opioid, methadone. Groups (N=8) of male and female rats first received subcutaneous injections of saline, methadone (4 mg/kg), or heroin (1 mg/kg), in a counter-balanced order. Tail withdrawal tests were performed 35 minutes after drug injections and again at 60, 90, and 120 minutes post-injection. Animals next underwent 30-minute sessions of exposure to PG, methadone (100 mg/mL), or heroin (100 mg/mL), in a counter-balanced order. Upon vapor clearance (5 minutes), rats were removed from the chambers and a tail withdrawal measurement was performed immediately, and again 60, 90, and 120 minutes, after the start of inhalation. These studies were performed with a minimum of three days between test sessions. Experimenters performing the tail-withdrawal were blinded to treatment conditions for both the passive and injection studies.

For self-administration rats, a pre-session baseline tail withdrawal assessment was performed immediately before the first self-administration session, i.e., prior to any drug

exposure. After commencement of self-administration training, a nociception assessment was performed prior to, and immediately after, the eighth one-hour session in both Cohorts. A second set of nociception assessments were performed on C2 prior to, and after, the 20th session. The duration for this latter self-administration session was reduced to 30 minutes to constrain the variability in drug intake pattern and the corresponding variability in drug distribution and metabolism. Start times for self-administration sessions for each rat were offset by five minutes to allow for the time required to complete the tail withdraw measurement for each rat. Experimenters performing the assay were blind to how many vapor deliveries a given rat had obtained.

A separate group of rats trained to self-administer intravenous (i.v.) heroin were included in this study to assess the similarity in degree of anti-nociception between i.v. heroin and inhaled heroin vapor. This group consisted of male Wistar rats that had been exposed to PG vehicle vapor or 100 mg/mL THC in PG vapor during adolescence and trained as adults to intravenously self-administer oxycodone in 8 h session (Nguyen et al., 2020). They had been switched to heroin IVSA for this study. For this experiment, a pre-session tail withdrawal assessment was performed which was followed by a 30-minute heroin i.v. self-administration session at a dose of 0.006 mg/kg/infusion. Withdrawal latencies were again measured immediately after the session.

2.4.2 Self-Administration Acquisition

Training began one day following completion of the two-week acclimation period to the vivarium. Behavioral testing was performed in a behavioral procedure room under red lighting. Vapor self-administration chambers were housed within an opaque black holding rack which remained closed throughout testing. Chambers were thoroughly cleaned, and bedding was replaced after each session. Upon initiation of a session, rats had access to two levers located on the right and left wall at the end of the chamber away from the door. Pressing the active drug-associated lever produced a one-second vapor delivery and illuminated a light above the lever. Each delivery was followed by a 20-second time-out period during which the light remained on and both levers were inactive. Following the time-out period, the chamber light turned off and the drug-paired lever once again became active. Pressing the non-paired inactive lever at any time throughout the session was recorded but was without consequence. Any active-lever presses, including those occurring during time-out periods, were recorded.

Animals initially underwent 10 one-hour FR (1) sessions with the drug at a concentration of 50 mg/mL heroin in the PG. The reinforcement contingency was then increased to an FR (2) schedule, which remained until completion of the study, and all rats underwent three more one-hour sessions to complete acquisition

Cohort 1: Animals in C1 completed the study after completing the 13 self-administration training sessions and testing in the concentration substitution experiments described below.

Cohort 2: After completing acquisition, rats in C2 continued for six more one-hour sessions, one 30-minute session immediately before a nociception assessment, and seven two-hour sessions. Concentrations were then substituted over 45 two-hour sessions. During this time, heroin was delivered at a concentration of 25 mg/mL across eight sessions and at 1 mg/mL over six. PG vehicle was given over the next 13 sessions, and the concentration was then increased back to 50 mg/mL for six sessions. Separate THC-heroin experiments were conducted across the following 12 sessions (data not shown). There was a four-day washout period before rats returned to the 50 mg/mL heroin training concentration for the EPM testing phase (described below). Animals then proceeded to substitution experiments followed by challenge with the antagonist naloxone.

2.4.3 Concentration substitution

Following initial acquisition training, rats in C1 underwent two-hour sessions on an FR (2) reinforcement contingency during which drug concentrations were altered (1 mg/mL, 50 mg/mL, and 100 mg/mL) for each session, in a counter-balanced order with every rat exposed to each concentration once. Animals in C2 were assessed following EPM testing. Drug concentrations (1 mg/mL and 100 mg/mL) were altered for each session, in a counter-balanced order and rats were exposed to each concentration once. Rewards for the 50 mg/mL concentration were calculated as the mean rewards obtained across the last two two-hour self-administration sessions conducted during the EPM testing phase. These two 50 mg/mL sessions were the last consecutive sessions prior to substitution experiments that were not conducted after any breaks in testing. Dependent variables for this experiment were number of reinforcers obtained and the number of active lever presses during the time-outs, as an indicator of the strength of drug seeking-behavior.

2.4.4 Naloxone challenge

Rats in C2 were returned to self-administration using the training concentration (50 mg/mL in PG vehicle) for one session following substitution experiments. Naloxone challenges were then performed over the next four sessions. Naloxone was administered via intraperitoneal injection five minutes prior to the session at doses of 0.0 mg/kg (saline), 0.03 mg/kg, 0.3 mg/kg, and 1.0 mg/kg at a volume of 1 mL/kg in saline, in a counter-balanced order. The challenge session was set to a total duration of 120 minutes and measurements were analyzed as 15-minute bins across the session, due to the rapid pharmacokinetics of naloxone. Baseline was calculated as the mean rewards obtained across the last two two-hour self-administration sessions conducted during the EPM testing, segmented into 15-minute bins.

2.5 Elevated plus maze test (EPM)

To investigate anxiety-like effects of drug discontinuation, rats in C2 were returned to self-administration (as described in acquisition methods) for two two-hour sessions and a one-hour session which was immediately followed by the first EPM trial. The one-hour session duration was chosen to minimize the time between the initial loading phase, where much of the exposure takes place, and testing. The following day rats underwent a two-hour self-administration session, and the second EPM test was performed 24 hours after completion of this session. Three more two-hour self-administration sessions were completed over three days and the third EPM test was conducted 48 hours after completion of the last session. Rats were motion-tracked during testing using AnyMaze software (Stoelting, Wood Dale, IL). Duration of EPM testing was set to 300 seconds. The training concentration (50 mg/mL heroin in PG) was used for the self-administration sessions in this study and the start times of self-administration sessions were offset across individuals to control for time gaps between the end of the session and the beginning of EPM testing.

2.6 Data analysis

Data were analyzed by Analysis of Variance (ANOVA) with repeated measures factors for Session in acquisition, Time after exposure in EPM experiments and in nociception assays, and for Concentration or Dose in substitution and naloxone experiments; a paired t-test was used when only two levels of a single factor were included in an analysis. Pre- and post-session

tail withdrawal data collected from rats in C2 following a 30-minute heroin vapor self-administration session were analyzed by paired t-test, and strength of association between tail withdrawal latency and reinforcers obtained was determined by Pearson correlation. A median split analysis of the acquisition data was planned based on pilot work with the inhalation model, as well as significant differences observed in intravenous self-administration studies (Creehan et al., 2015; Vandewater et al., 2015; Nguyen et al., 2018). Median splits were determined within each Cohort by ranking individuals on mean number of reinforcers obtained across the 10 sessions of acquisition. The upper half is termed High Responder and the lower half is termed Low Responder. Significant effects were followed with post-hoc analysis using the Holm-Sidak procedure. A criterion of $p<0.05$ was used to infer significant effects. Analysis were performed using Prism 8 for Windows (GraphPad Software, Inc, San Diego, CA).

3. Results

3.1 Nociception assay

Non-contingent administration of heroin or methadone via injection or vapor delivery increased tail withdrawal latency (**Figure 1**). Preliminary analysis confirmed there was no significant sex-difference, or interaction of drug conditions with sex, thus the data were collapsed across sex for final presentation and subsequent analysis. The ANOVA for the injection study confirmed significant effects of Drug Condition ($F(2, 45) = 27.74$, $P<0.0001$), of Time post- injection ($F(3, 135) = 105.8$, $P<0.0001$) and of the interaction of Time and Drug Condition ($F(6, 135) = 28.20$, $P<0.0001$) on tail withdrawal latency. Similarly, the ANOVA for the inhalation study confirmed significant effects of Drug Condition ($F(2, 30) = 7.302$, $P=0.0026$),of Time after the start of inhalation ($F(3, 45) = 26.89$,

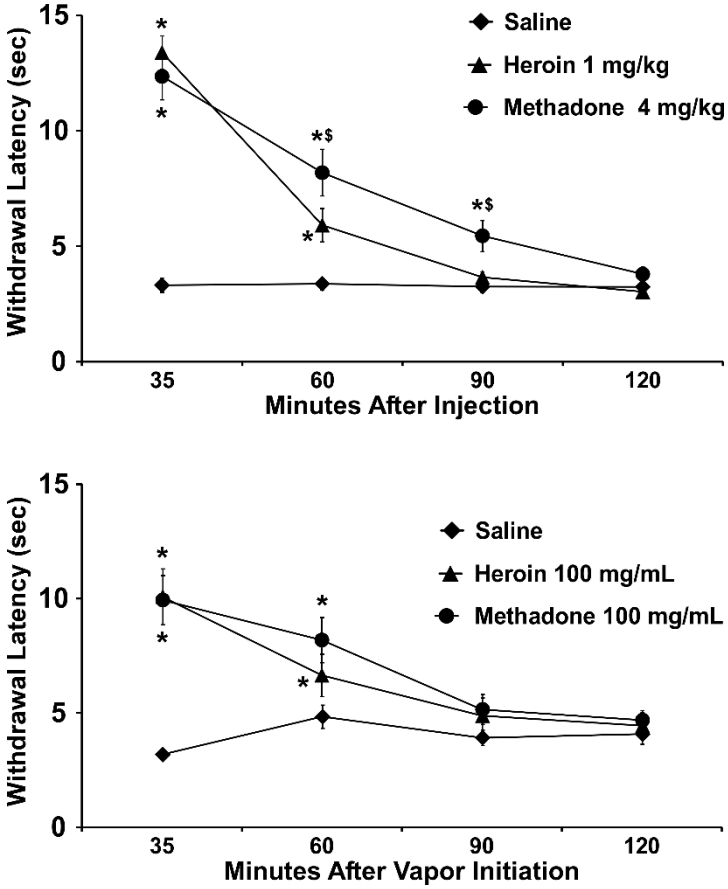

***Figure 1**: Mean (N=16; ±SEM) tail withdrawal latencies following (top) injection or (bottom) inhaled routes of administration for heroin or methadone. A significant difference from vehicle (Saline or PG, respectively) at a given time point is indicated with * and from all other treatment conditions with $.*

P<0.0001) and of the interaction of Time and Drug Condition (F (6, 90) = 11.13, P<0.0001) on tail withdrawal latency.

3.2 Acquisition of self-administration of vaporized heroin

Rats were trained to voluntarily lever-press for contingent vapor deliveries, and consequently exposed themselves to approximately 5-10 minutes of heroin vapor inhalation during the initial 10 session (**Figure 2A**). Analysis of active lever discrimination for the FR(1) sessions confirmed only a main effect of Session (F(9,126)=4.301, p<0.0001; **Figure 2B**). Animals exhibited higher active lever discrimination on sessions 8 and 10 compared with session 1, and higher discrimination on session 4, 8, and 10 compared with session 2. No significant effects were confirmed for active lever discrimination for the FR(2) sessions. Analysis of reinforcers, by median split, confirmed a main effect of Group (F(1,14)=8.972, p<0.01; **Figure 2C**), with High Responders (HR) self-administering higher numbers of reinforcers compared to Low Responders (LR). Analysis of reinforcers obtained during the subsequent three FR(2)

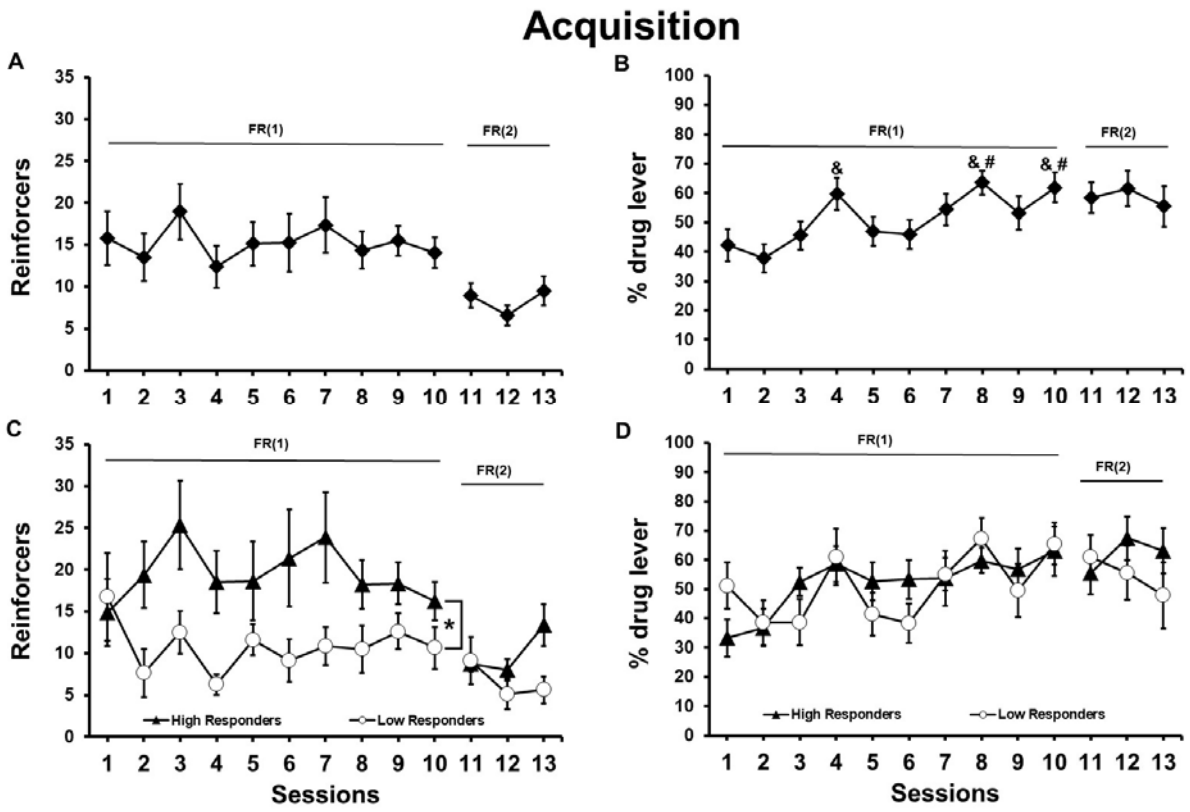

***Figure 2**: Mean (N=16; ±SEM) A) reinforcers (30 sec epochs of vapor delivery) and B) lever discrimination ratios for heroin vapor self-administration female rats for High and Low responder sub-groups (C, D). Significant differences from LR condition are indicated by *. Significant difference from session 1 and session 2 are indicated by # and &, respectively.*

sessions by median split (**Figure 2 C**) did not confirm a significant effect of Group, of Session or of the interaction (p=0.056) despite a recovery in the more-preferring HR rats in Session 13.

3.3 Concentration substitution

Changes in the heroin concentration in the vapor solutions altered the drug seeking behavior of the rats (**Figure 3**). HR (N=8) and LR (N=8) sub-groups were analyzed separately for these experiments. Within the HR group, a significant main effect of Concentration was confirmed for reinforcers obtained ($F(2,14)=6.462$, $p<0.05$; **Figure 3A**). Post hoc analysis confirmed a significant increase in reinforcers obtained for the 1 mg/mL heroin concentration compared with 100 mg/mL heroin concentrations. No effect of Concentration was confirmed

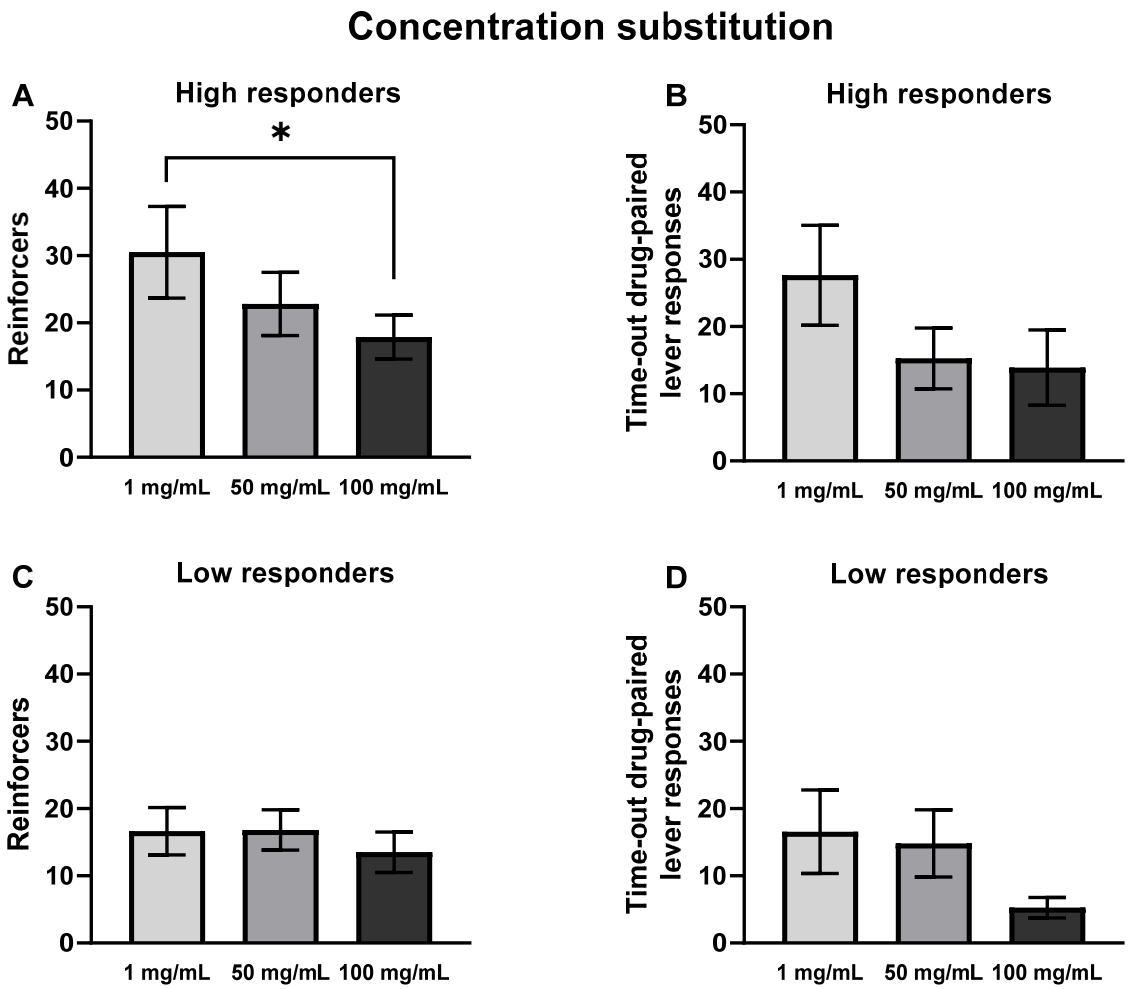

***Figure 3**: Mean (±SEM) reinforcer deliveries (A,C) and time-out drug-associated lever responding (B,D) for High (A, B) and Low (C, D) Responder sub-groups. A significant difference between concentrations is indicated with *.*

within the HR group for time-out drug-associated lever responding (p = 0.05; **Figure 3B**). The mean number of reinforcers obtained by the LR rats did not differ across concentrations (**Figure 3C**) and time-out responses were not statistically different (p = 0.076; **Figure 3D**).

3.4 Naloxone challenge

Pre-treatment with the mu opioid receptor antagonist increased the number of reinforcers obtained by the HR rats, in a dose-dependent manner (**Figure 4**). Reinforcers obtained in the first 15 minutes of the session by HR and LR rats (N=4 per treatment condition) were analyzed separately for these experiments and the data are presented as % of baseline to illustrate an apparent effect of the pre-session injection protocol. The ANOVA confirmed a significant effect of Dose ($F(3,9)=12.81$, $p<0.01$) on reinforcers obtained by the HR rats. The post hoc analysis confirmed a significant increase in the 0.3 mg/kg dose condition, and a decrease in the 1.0 mg/kg dose condition, in comparison with saline pre-treatment. No significant effects of pre-treatment condition were confirmed in the LR group.

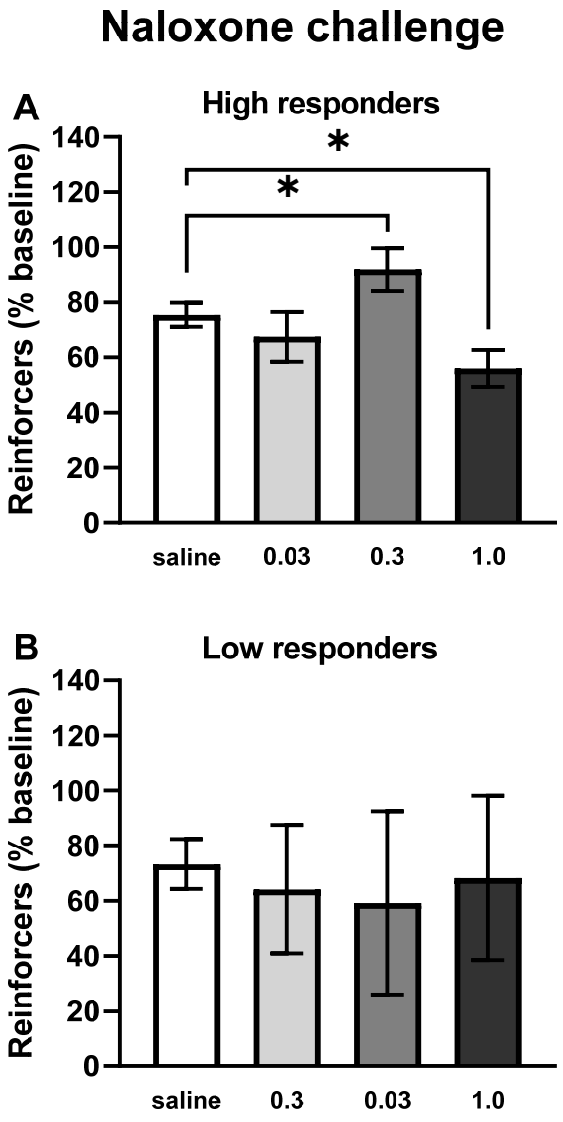

Figure 4: *Mean (±SEM) reinforcers obtained by HR (A; N=4) and LR (B; N=4) sub-groups of Cohort 2 following i.p. naloxone injection.*

3.5 Anti-nociceptive effects of self-administered heroin.

Tail withdrawal latencies were recorded prior to any drug exposure, and before and after the eighth self-administration session for rats in both cohorts (**Figure 5A**). There was no significant difference in tail withdrawal latency between HR and LR groups, thus

the data were pooled. The analysis confirmed a significant effect of Timepoint ($F(2,28)=15.14$, $p<0.0001$) on tail withdrawal. Post hoc analysis confirmed that tail-withdrawal latency was significantly longer post-session compared with either baseline and pre-session measurements. No differences were detected between baseline and pre-session time points. A second tail withdrawal test was performed on rats in C2 prior to and after the 20th self-administration session, which was shortened to 30 minutes duration to constrain variability in intake pattern. Because no effect of preference sub-group was detected in the first tail withdrawal assay, these data were analyzed as one group. A paired t test confirmed a significant difference between the pre- and post-session measurements ($t(7)=2.921$, $p<0.05$; **Figure 5B**); post-session withdrawal latencies were significantly higher compared with pre-session. A Pearson correlation also confirmed a significant positive association between the number of reinforcers obtained during

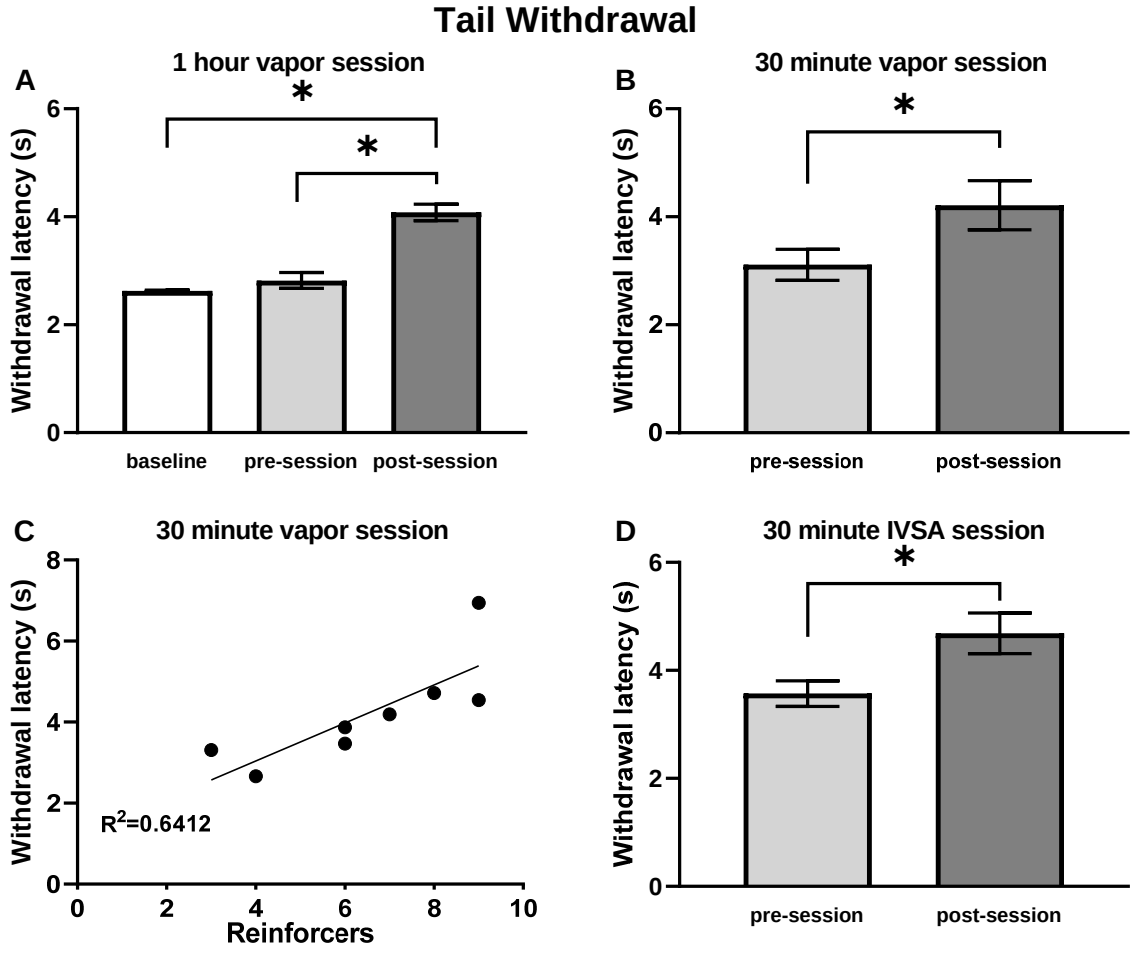

Figure 5: *Mean (±SEM) tail withdrawal latency for A) Cohorts 1 and 2 (N=16); B) Cohort 2 (N=8) rats before and after a 30 min heroin vapor session; C) Correlation of individual (N=8) withdrawal latency with the number of reinforcers obtained in the prior self-administration session for the 30 min vapor inhalation experiment; D) Male Wistar (N=8) rats trained in intravenous heroin self-administration and assessed before and after the session. A significant difference between pre-/post-session is indicated with *.*

the self-administration session and tail withdrawal latency in the 30 minute experiment ($r=0.8008$, $p<0.05$; **Figure 5C**).

Analysis of tail withdrawal latency data from a separate group of male rats that were permitted to self-administer heroin intravenously for 30 minutes (**Figure 5D**) confirmed a significant main effect of time of assessment ($F(1,15)=8.012$, $p\leq0.01$). There were no main effects of the prior adolescent repeated THC exposure or interactions of adolescent exposure with the time of assessment confirmed. Therefore, post-session withdrawal latencies were significantly higher after heroin IVSA compared with pre-session latencies, irrespective of adolescent drug exposure.

3.6 Elevated plus maze

The time rats spent in the open and closes arms of the EPM varied depending on the interval of time since last drug access (**Figure 6**). The statistical analysis of the EPM behavior confirmed a significant effect of the post-session interval for time spent in the closed arms ($F(2,14)=6.233$, $p<0.05$; **Figure 6A**) and time spent in the open arms ($F(2,14)=7.030$, $p<0.01$; **Figure 6B**) of the maze. The post hoc analysis confirmed an increase in time spent in the closed arms at the 48-hour time point compared with the immediately post-session assessment. The post hoc analysis also confirmed a significant decrease in the time spent in the open arms at both the 24- and 48-hour post-session time points compared with the immediate post-session time point. Analysis of arm entries also confirmed significant effects of drug discontinuation time for entries into the open arms ($F(2, 14)=7.366$, $p<0.01$; **Figure 6D**) and the post-hoc test confirmed fewer mean entries 24- and 48-h after last drug availability compared with the immediate post-session assessment. No effects of time since last drug availability were confirmed for closed arm entries.

4. Discussion

In the present study, heroin vapor generated by an Electronic Nicotine Delivery System (ENDS; “e-cigarette”) technology reinforced operant responding, in a manner that is consistent with heroin self-administration. This extends a prior result using extended daily access sessions (12 h) and the high potency opioid sufentanil (Vendruscolo et al., 2018) to the less-potent, but

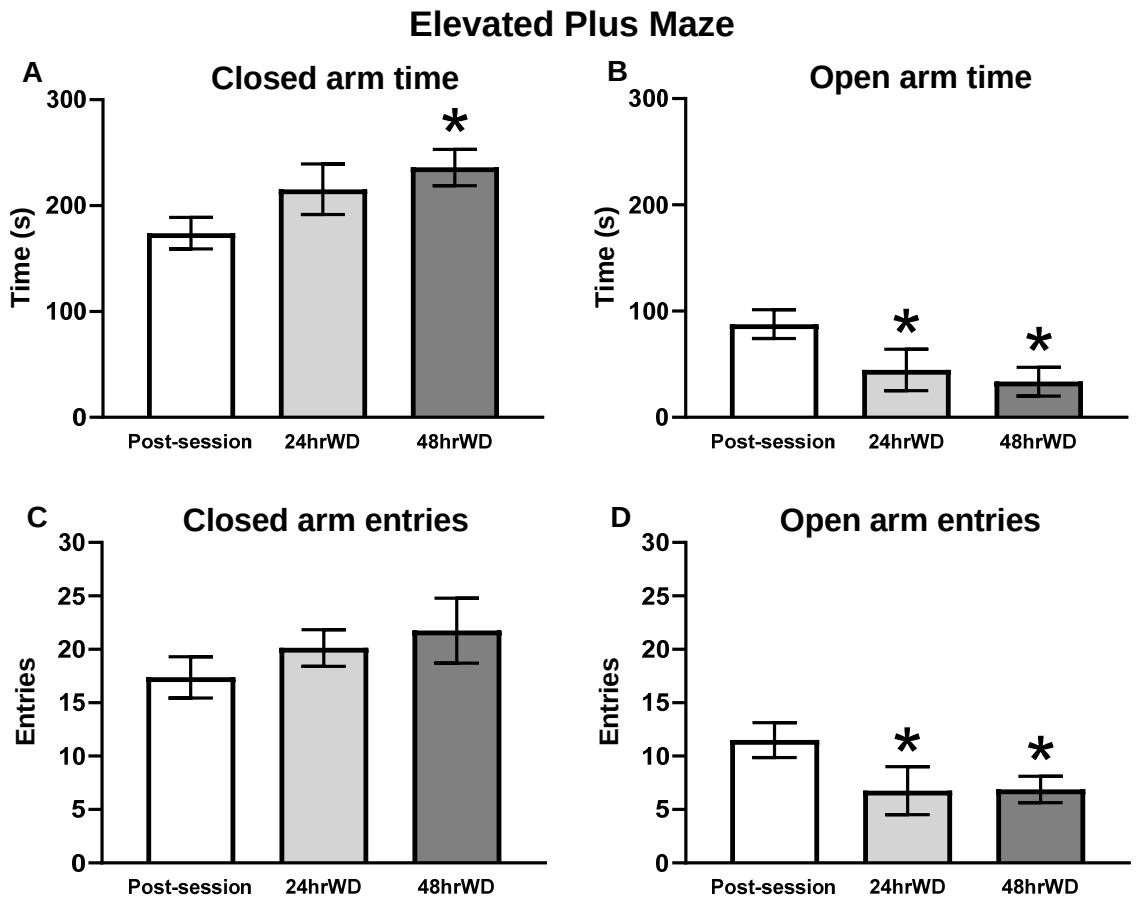

Figure 6. *Mean (N=8; ±SEM) time spent in A) closed and B) open arms, and number of entries into the C) closed and D) open arms of the elevated plus maze. A significant difference between assessment times, relative to the last self-administration session, is indicated with *.*

more commonly abused, opioid heroin, to short-access sessions and to female rats. The first major finding of this study was the demonstration that non-contingent delivery of less potent opioids such as heroin and methadone by ENDS technology produced significant analgesia in rats. Thus, this approach can be useful for the assessment of a wide range of effects of several common opioids when delivered by vapor inhalation in rats. The second major finding of this study was that the higher preference rats met two key criteria for self-administration, i.e. they compensated for reductions in the heroin concentration in the vapor with increased behavioral responding, and they similarly compensated for the effects of a moderate dose of the opioid receptor antagonist naloxone. Furthermore, acute intoxication via vapor self-administration was shown to produce anti-nociception comparable to that produced by intravenous self-administration of heroin, confirming a similarity in the self-determined heroin intake level by the

two routes of administration. The fact that individual differences in anti-nociception correlated with the number of vapor deliveries obtained in the prior self-administration session provides converging evidence on the function of the model. Lastly, anxiety-like behavior, assessed in an EPM, increased following 24 and 48 hours of drug discontinuation, suggesting that withdrawal-like effects develop shortly after access to heroin vapor is withheld, similar to what is observed after access is withheld in an intravenous self-administration model.

In the concentration-substitution experiment the high responder group was self-administering ~20 vapor deliveries and were sensitive to a reduction in the heroin concentration they increased the number of obtained reinforcers to compensate. The time-out responding on the drug-associated lever also increased, again suggestive of enhanced drug-seeking associated with the lower available concentration of heroin. The low responders did not increase responding for lowered concentrations and only reduced the time out responding at the higher concentration of heroin. This pattern is potentially consistent with an interpretation that this subgroup was more sensitive to aversive properties of higher levels of intoxication. This may explain why they were low, but continued responders, i.e., no rats in the group ever extinguished their responding. The reduction in drug-seeking behavior (indexed by time-out responding) observed in the lower preference sub-group at the highest concentration dose-substitution might therefore be viewed as evidence of overall purposeful, drug-motivated behavior on the part of the lower-preferring individuals.

Treatment with an antagonist such as naloxone can dose-dependently affect responding for opioid drugs, enhancing response at lower doses and suppressing responding as the dose is increased. For example, naloxone given at 1 mg/kg suppressed responding for aerosolized sufentanil in rats (Jaffe et al., 1989) and smoked heroin in rhesus monkeys (Mattox and Carroll, 1996), and increased responding for intravenously administered heroin in rats when given at doses ranging from 0.003 to 0.3 mg/kg (Carrera et al., 1999; Chen et al., 2006). For the higher preference subgroup in this study, naloxone altered vapor self-administration in an inverted U dose-effect pattern suggesting that the intermediate dose attenuated heroin reward whereas the higher dose may have prevented it entirely under these inhalation conditions. This further supports the conclusion that rats were responding to self-administer an individually determined dose of heroin via inhalation.

In the nociception assays conducted after self-administration, the tail-withdrawal latencies were increased, consistent with the anti-nociceptive properties of heroin. The effect size was not

large compared with the effects of larger parenteral injection of heroin (Tasker and Nakatsu, 1984), methadone (Holtman and Wala, 2007) or oxycodone (Nguyen et al., 2019b), or a non-contingent delivery of methadone or heroin vapor (generated from 100 mg/mL) for 30 minutes (Figure 1). Some degree of tolerance due to the repeated self-administration might be expected, for example, tolerance to anti-nociceptive effects of a 1 mg/kg, s.c. dose of oxycodone is induced by an interval of intravenous oxycodone self-administration, as we previously showed (Nguyen et al., 2018). Most importantly, we show here that the magnitude of anti-nociception produced by self-selected dosing of either inhaled or i.v. heroin is identical. This supports the conclusion that rats are self-administering to a similar degree of intoxication in an inhalation session as they do when *intravenous* infusions are made contingent upon a lever press.

Despite the fact that this was a short-access (2 h) model, rats did exhibit signs of increasing anxiety-like behavior across 24 h and 48 h of drug abstinence using the elevated plus-maze approach. This is, again, consistent with results for discontinuation from intravenous self-administration of heroin. As a minor caveat, in this study, EPM testing was performed over repeated trials for each individual, in contrast with the more generally adopted approach of testing only a single trial per individual rat. A major concern of repeated trials has been that habituation to the testing environment may occur over time and therefore reduce test sensitivity. This may not always be the case, however, since the stability of EPM testing over time has been reported in non-treated rats (Schrader et al., 2018). Nevertheless, we took a cautious approach in the EPM assay by performing testing in ascending order of drug discontinuation time. If habituation to the apparatus did indeed occur, it would be expected that open arm time and entries would increase across successive EPM assessments. The opposite was observed, however; rats further decreased open arm time and entries as the interval of drug withholding was increased. Whether some degree of habituation occurred over time but was masked by the magnitude of the withdrawal expression cannot be determined from the present study. Resolution would require additional parallel groups to fully determine. For the current purposes, assessing habituation or the precise quantitative amount of anxiety-like behavior was not the primary aim of the study. Since anxiety-like effects were detected, irrespective of previous exposure to testing environment, it may be concluded that, if anything, the degree of withdrawal was underestimated in this design.

Caveats and Future Directions for ENDS Based Self-Administration Models

As reviewed above there have been a few prior self-administration studies published, using inhalation of methamphetamine, cocaine or heroin, in an attempt to mimic human vapor inhalation. The methodological rigors and the need to manufacture delivery systems within laboratory has apparently been a barrier to broad use. The wide availability of ENDS type devices and the relative ease of delivering active doses of a range of psychoactive drugs to laboratory rodents using them has reinvigorated interest in vapor self-administration models. One recent report may even show rat self-administration of cannabis extracts (Freels et al., 2020), a class of drugs for which convincing evidence of rodent self-administration by any route is thin at best. The present evidence for ENDS self-administration of drugs is promising in that rats will continue to respond for drug-infused vapor, session after session, and exhibit some of the usual criteria such as withdrawal-like effects, dose sensitivity and antagonist sensitivity.

One clear limitation in this model, relative to conventional IVSA criteria for self-administration, is the relatively low ratio of responses on the drug-associated versus the non-associated lever. Relatedly, the rats tended to make a lot of drug-associated lever responses during the time-out interval. This result in our study mirrors similar patterns reported by Freels and colleagues and by Vendruscolo and colleagues (Vendruscolo et al., 2018; Freels et al., 2020), thus it may be a commonality of vapor self-administration. Another limitation is the distribution of individual differences. Similar differences have been reported for the acquisition of intravenous self-administration of 3,4-methylenedioxymethamphetamine (Schenk et al., 2007; Vandewater et al., 2015) and even for cocaine if a subthreshold training dose is used (Smith and Pitts, 2011).

It should be recognized that the first rodent morphine intravenous self-administration study was reported in 1962 by Weeks, (Weeks, 1962), followed by evidence for other opioids (Collins and Weeks, 1965) and then heroin (Blakesley et al., 1972). Since this time there have been a large number of studies published using IVSA to evaluate drug reinforcement in monkeys, rats and mice, including over 1,000 for heroin alone. There are undoubtedly many additional variants of the inhalation approach to explore which may turn out to have advantages and disadvantages relative to the current model. This should be viewed as an initial demonstration of feasibility and a departure point for examining other variations in approach, which may have improved utility across a range of experimental goals.

5. Conclusions

These studies show that self-administration of heroin by vapor inhalation in rats using ENDS technology can be produced in short-access models, complementing prior observation of the establishment of vapor inhalation of the highly potent opioid sufentanil using extended daily access sessions (Vendruscolo et al., 2018).

Author Contributions:

AG, JDN and MAT designed the studies. AG, KMC and JDN performed the research and conducted initial data analysis. The authors are grateful to Sophia A. Vandewater for assistance with blinding of the initial anti-nociception experiment. AG conducted statistical analysis of data, created figures and AG and MAT wrote the initial draft of the paper. All authors approved of the submitted version of the manuscript.

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