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Determining impacts of prenatal cannabis exposure on cannabis vapor self-administration using a novel response-contingent vapor model in pregnant rat dams

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ABSTRACT

Cannabis use during pregnancy is becoming increasingly common despite a lack of knowledge regarding its long-term effects on developing offspring. Determining effects of prenatal cannabis exposure on cannabis use later in life has been especially difficult given the problems inherent to traditional models of cannabinoid self-administration. Thus, we adopted a model of response-contingent cannabis vapor delivery in pregnant rat dams to investigate impacts of maternal cannabis use on reinforcing properties of Δ^9-tetrahydrocannabinol (THC)-rich cannabis vapor in offspring. Rat dams were trained to self-administer a vaporized cannabis extract or vehicle vapor in 1-hr sessions twice daily until 24-48 hr prior to parturition, while a third group received no vapor exposure. Cannabis vapor self-administration was assessed in adult offspring using a 22-day escalating reinforcement schedule that culminated in a 3 hr progressive ratio challenge. Dams reliably self-administered cannabis vapor during the gestational period and showed better discrimination for the vapor-paired nosepoke than vehicle self-administering dams. In accordance with human data, cannabis-exposed offspring displayed lower birthweights than vehicle-exposed offspring. Effects of prenatal cannabis exposure on vapor self-administration in adult offspring differed by sex. Male cannabis-exposed offspring made fewer active responses and earned fewer vapor deliveries than vehicle-exposed offspring, regardless of their assigned vapor condition. Conversely, female offspring showed higher rates of responding for cannabis relative to vehicle, but rates of self-administration were unaffected by prenatal cannabis exposure. Altogether, these data demonstrate feasibility of response-contingent cannabis vapor delivery in pregnant rat dams and indicate paradoxical suppressive effects on vapor self-administration in male offspring.

1. Introduction

As the number of states with legal cannabis continues to increase, there has been a concomitant rise in cannabis use among pregnant women [8]. Approximately 4-7% of pregnant women report using cannabis [8,17,42,49], while rates of past-month cannabis use have increased 62% from 2002 to 2014 [8], particularly among young, urban, low-income individuals [41]. Furthermore, medicinal cannabis use is becoming more prevalent in pregnant women because of its antiemetic properties [29,47]. Human longitudinal studies have offered valuable insight into the long-term effects of maternal cannabis use on developing offspring. However, co-morbid or pre-existing conditions can often complicate interpretation of these data [41]. Accordingly, very little is known about the enduring impacts of maternal cannabis use. Given that the prevalence of maternal cannabis use is expected to increase in the coming years [46], there is an urgent need to better understand the long-term effects of prenatal cannabis exposure.

Animal research has provided a powerful tool to investigate effects of cannabis and other drugs on neurodevelopment. However, studies exploring effects of maternal cannabis use typically use synthetic cannabi-

Abbreviations: THC, Δ^9-tetrahydrocannabinol; 11-OH-THC, 11-hydroxy-Δ^9tetrahydrocannabinol; P, postnatal day; CBG, cannabigerol; CBD, cannabidiol; CBC, cannabichromene; Δ^8-THC, Δ^8-tetrahydrocannabinol; THCV, tetrahydrocannabivarin; VEH, vehicle; FR, fixed ratio; PR, progressive ratio; THC-COOH, 11-nor-9-carboxy-THC; ANOVA, analysis of variance; ANCOVA, analysis of covariance; LSD, least significant difference.

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noids or isolated Δ^9-tetrahydrocannabinol (THC) preparations, which are pharmacologically distinct and do not consider potential effects of the many other phytocannabinoids present in whole-plant preparations [41]. Additionally, the most common route of cannabis administration for humans is inhalation, which differs pharmacokinetically from the methods traditionally used in animal studies [3,21]. This is important because studies comparing injected vs. vaporized THC have revealed markedly different pharmacokinetic profiles despite producing equal peak concentrations of circulating THC [3]. Specifically, vapor delivery produces significantly higher initial brain THC concentration, whereas injection produces higher concentrations of the psychoactive metabolite 11-hydroxy-THC (11-OH-THC) in both blood and brain that further increased over time [3]. In pregnant rats, repeated exposure to vaporized THC vapor produced fetal brain THC concentrations that are about 30% of those seen in maternal blood, whereas repeated THC injections produced roughly equivalent concentrations of THC in maternal blood and fetal brain [4]. Together, these discrepancies may result in different effects in animal models that may not be evident in human clinical populations [21].

To address this issue, our laboratory has developed and validated a novel approach that allows rats to self-administer vaporized whole-plant cannabis extracts in a response-contingent manner [14,15], thereby providing a more translationally valid model for studying the effects of cannabis use. The vapor self-administration model produces biologically relevant plasma concentrations of THC and its metabolites, and elicits a metabolic and behavioral phenotype that is consistent with effects of cannabis use in humans [14]. Importantly, our recent studies have indicated that female rats reliably self-administer cannabis vapor at significantly higher rates than male rats [15]. Given the propensity for female rats to respond for cannabis vapor in this model, we sought to determine whether pregnant rat dams would continue to self-administer cannabis vapor at physiologically relevant rates throughout the gestational period.

Research in human populations indicates that cannabis use during pregnancy is associated with use of cannabis in offspring [9,12,27,34]. Maternal cannabis use during pregnancy is associated with earlier initiation of cannabis use in exposed offspring [9]. Adolescents and young adults who were exposed to cannabis *in utero* are also more likely to use cannabis than their peers [9,12,27,34]. Cannabis-exposed adolescents also use cannabis more frequently than their age-matched counterparts [9]. Thus, human studies suggest that prenatal cannabis exposure may be a significant vulnerability factor that increases the risk for problematic cannabis use later in life. Animal studies indicate that prenatal cannabinoid exposure increases seeking for alcohol [7], heroin [35], and morphine [40]. However, no animal studies to date have examined whether prenatal cannabinoid exposure predisposes offspring to later *cannabinoid* use.

In the current study, we adapted the vapor self-administration model [14,15] to allow pregnant rat dams to self-administer cannabis throughout the gestational period. We then examined rates of cannabis vapor self-administration in their offspring during adulthood. We hypothesized, based on the human literature, that cannabis vapor self-administration during pregnancy would increase preference and motivation for cannabis vapor in the adult offspring. Contrary to expectations, our data indicate that maternal cannabis self-administration does not increase motivation for cannabis vapor in offspring, but rather led to *reduced* rates of vapor self-administration, particularly among male cannabis-exposed offspring.

2. Materials and Methods

2.1. Animals

Nulliparous female Sprague Dawley rats (50 days old; Envigo, Indianapolis, IN) were pair-housed and randomly assigned to either the cannabis, vehicle, or no vapor treatment group. Following one week of handling and habituation, rats assigned to the vapor groups began self-administration. After self-administration acquisition, female rats underwent a 10-day period of mating and then were single housed until giving birth. The day of birth was designated postnatal day (P) 0. Offspring were weaned into same-sex housing on P21. Vapor self-administration began when offspring reached approximately postnatal day (P) 90-100. All rats lived in a humidity-controlled animal suite on a 12 hr on-12 hr off reverse light cycle with food and water available *ad libitum*. All procedures were performed in accordance with the guidelines in the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Washington State University Institutional Animal Care and Use Committee.

2.2. Drugs

Raw whole-plant cannabis extract was provided by the National Institute on Drug Abuse (NIDA) Drug Supply Program. Based on the provided certificate of analysis, the extract contained 69.81% THC, 2.69% cannabigerol (CBG), 1.51% cannabinol (CBN), 0.89% tetrahydrocannabivarin (THCV), 0.83% cannabichromene (CBC), 0.73% Δ^8-tetrahydrocannabinol (Δ^8-THC), and no detectable cannabidiol (CBD). To separate phytocannabinoids from other raw plant material, extract was heated overnight at 60˚C in ethanol under constant stirring. Once the ethanol evaporated off completely, extract was centrifuged at 4000g for 15 min. Extract containing phytocannabinoids was then collected and diluted to 150 mg/mL cannabis extract in 80% propylene glycol/20% vegetable glycerin vehicle. This dose was chosen based on observed rates of vapor self-administration at several concentrations of cannabis extract previously established in our laboratory [15].

2.3. Vapor Self-Administration

Vapor self-administration was conducted using a vapor delivery system running MED-Associates IV (Fairfax, VT) or La Jolla Alcohol Research Inc. software (LJARI; La Jolla, CA). Each vapor chamber (14.5" L x 10.5" W x 9.5" H; Fig. 1A) contains one active and one inactive nosepoke operandum. When a beam breaks in the active nosepoke, a signal is sent to the computer to release a 3 sec puff of vapor, a cue light illuminates, and a 1 min timeout period begins. Nosepokes on the inactive nosepoke result in no vapor release or cue light illumination. The vapor is generated by a commercial e-cigarette SMOK Baby Beast TFV8 tank with a 0.2Ω M2 atomizer, 40-60 W range (SMOKtech, Shenzhen, China) containing the assigned vehicle or cannabis preparation. The system is under continuous vacuum, pulling vapor into the chamber and out through an exhaust port at an exchange rate of 1.0 L air/min.

Following each session, the vapor self-administration program generated a total number of active nosepoke responses, inactive nosepoke responses, and vapor deliveries. These data were then used to calculate a discrimination index (DI) using the formula: $DI = \frac{active\ nosepokes - inactive\ nosepokes}{active\ nosepokes + inactive\ nosepokes}$. Discrimination index ranges between -1 and 1, where -1 indicates all responding on the inactive nosepoke, 0 indicates equal rates of responding on either nosepoke, and 1 indicates all responding on the active nosepoke.

Maternal self-administration occurred as depicted in Fig. 1B. To ensure dams were at stable rates of self-administration by conception, female rats in the vapor groups began acquisition ten days prior to mating. Dams in the no vapor group were weighed daily but otherwise remained in the animal suite for the duration of the study. Dams underwent twice daily, hour-long self-administration sessions in a vapor chamber room separate from the animal suite. The first session began two hours into the dark cycle and the second started two hours prior to the onset of the light cycle. The schedule of reinforcement was set to fixed ratio (FR)-1 such that each nosepoke in the active nosepoke not occurring during a timeout resulted in a vapor reinforcer. Self-administration continued until 24-48 hr prior to birth.

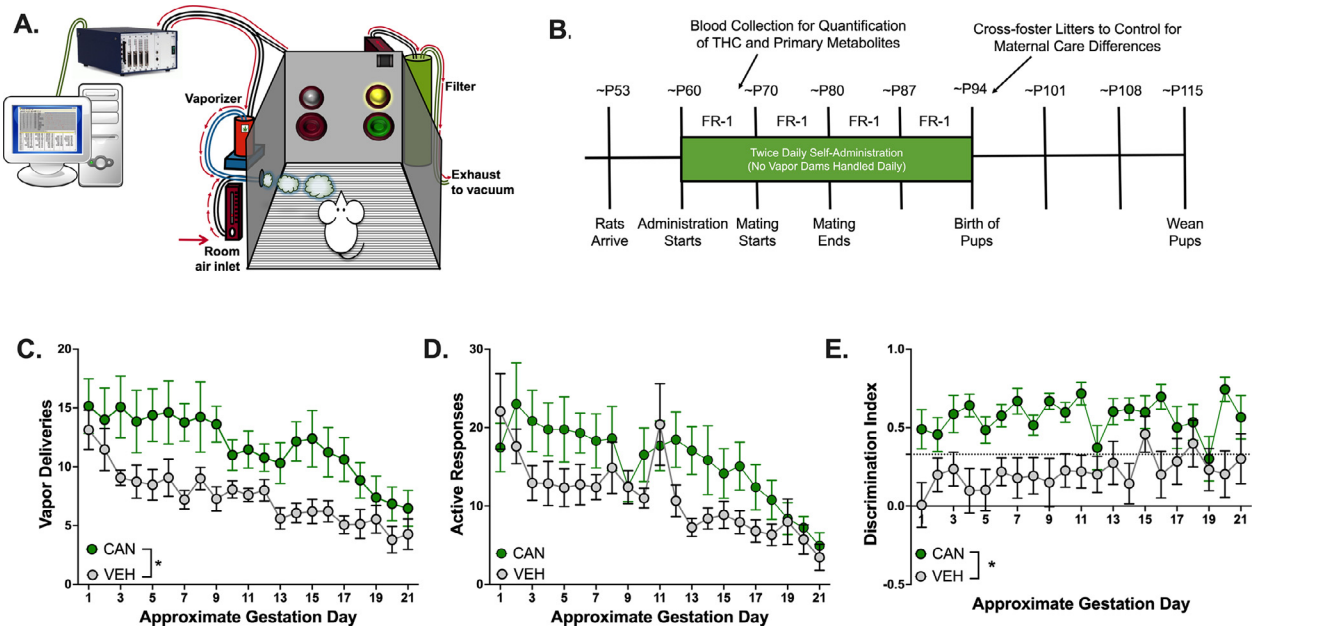

Fig. 1. ***Pregnant rats self-administer cannabis vapor at higher rates and show greater discrimination for the vapor-paired nosepoke.*** **(A)** Vapor chamber schematic. **(B)** Experimental timeline. **(C)** Dams that self-administered cannabis vapor earned more vapor deliveries than dams that self-administered vehicle vapor during the gestational period. **(D)** The number of daily active nosepokes for cannabis vapor or vehicle vapor was not significantly different over the gestational period (note: active nosepokes include responses made during the time-out period). **(E)** The daily discrimination index of dams that self-administered cannabis vapor was significantly higher than the discrimination index of dams that self-administered VEH vapor during the gestational period. The dotted horizontal line represents 2:1 active:inactive response ratio. * indicates significant differences between cannabis and vehicle vapor groups (p = .05). Values indicate mean +/- SEM. n=14-16 dams/treatment condition.

Offspring self-administration occurred as previously described [14]. Briefly, rats from each prenatal treatment were randomly assigned to administer either cannabis (150 mg/ml) or vehicle vapor. Rats self-administered their assigned vapor once daily on an FR-1 schedule for 11 days, and then advanced to an FR-2 schedule for 5 days, followed by an FR-4 schedule for the next 5 days. On the final day of testing, rats underwent a 3-hour progressive ratio (PR) challenge where the schedule of reinforcement increased according to programmed increments: 1, 1, 2, 2, 3, 3, 4, 4, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, 15, 15, 18, 18, 21, 21, 24, 24, etc. [14,43]. In addition to these measurements, the breakpoint in the PR challenge was calculated, which was the number of vapor deliveries earned before the rat entered a 15 min period of no responding.

2.4. Plasma Concentration of THC and Metabolites

Plasma concentrations of THC and metabolites were determined as described previously [3,4,15]. Blood was collected from the tail vein of rats in the cannabis group in sterile tubes containing 0.1 mL ethylenediaminetetraacetic acid. Blood collection occurred immediately following the vapor self-administration session on the day before mating so as not to introduce a stressor during the gestational period. Blood was centrifuged at 4°C at 4000g for 15 min. Plasma was extracted and stored at -20°C until transportation on dry ice to the Southern Alberta Mass Spectrometry (SAMS) facility at the University of Calgary, Calgary, CA. Prior to sample processing, an internal standard (IS; d3 analytes) was created by adding 10 ng/mL deuterated THC, 11-OH-THC, and 11-nor-9-carboxy-THC (THC-COOH) (Cerilliant, Round Rock, TX) to a 50% methanol/50% water solution. Fifty μL of thawed serum was pipetted into tubes containing 2 mL acetonitrile and 100 μL IS. These solutions were then sonicated on ice for 30 min, held at -20°C overnight, and then centrifuged at 4°C at 1800 rpm for 3-4 min. Supernatant was pipetted off and then evaporated with nitrogen gas. 250 μL of acetonitrile was added to the tube and then evaporated again with nitrogen gas. Once completely evaporated, 100 μL of a 50% methanol/50% water solution was added. This solution was centrifuged two full times at 4°C at 15,000 rpm for 20 min. Supernatant was pipetted into a glass tube containing a glass insert, and then the tubes were placed in the -80°C freezer. On the day of analysis, samples were run with a LC-MS/Multiple Reaction Monitoring (MRM) program using the positive electrospray ionization and multiple reaction monitoring mode. Analysis was performed on a Eksigent Micro LC200 coupled to AB Sciex QTRAP 5500 mass spectrometry (AB Sciex, Ontario, CA). Normalized analyte concentration for Δ^9-THC, 11-OH-THC, and THC-COOH was generated by accounting for sample volume.

2.5. Gestational Endpoints

During mating, female rats were checked daily for signs of copulation to estimate conception date. The birth and copulation dates were used to calculate gestation length. To assess changes in food intake as a consequence of maternal cannabis self-administration, pellets from the food hopper were weighed daily once females were single housed, through the day before birth. Maternal weight gain was determined by calculating the percent change from the weight on the day prior to giving birth and the weight on the estimated conception date.

2.6. Litter Endpoints

On P1, rat pups were weighed individually and as litters. Pups were counted to determine litter size. Pups were sexed to determine ratio of males to females. As differences in maternal care may impact later behavior, pups were cross fostered between available litters on P1. To distinguish prenatal treatment in mixed litters, pups were first tattooed with a small dot of Super Black^{TM} india ink (Speedball, Statesville, NC, USA) on either their left, right, or neither hindpaw. Ideally, we cross fostered litters such that each dam raised roughly 12 pups with each prenatal treatment group represented. In some cases, only litters from two treatments were available for cross-fostering on the same day. On rare occasions, dams raised pups from two litters sharing the same treatment due to no other litters being available for cross-fostering.

2.7. Statistics

Maternal self-administration data were analyzed via two-way mixed factorial analysis of variance (ANOVA). There was no difference in rates of vapor self-administration between morning and evening sessions – thus, these sessions were collapsed and analyzed by day. Only the last 21 days of self-administration data were analyzed, as they are considered to reflect use occurring specifically post-conception. Fisher's Least Significant Difference (LSD) test was used to further investigate differences *post hoc*.

One-way between-subjects ANOVAs were used to analyze most gestational endpoints and litter statistics. Chi square analysis was used to examine percent of rats who gave birth and percent of male and female pups in each litter. Two-way mixed factorial ANOVAs were used to investigate difference in body weights in offspring as P1 neonates and adults with Tukey's *post hoc* used to probe differences. To control for potential maternal care effects in the early neonatal period, two-way mixed factorial analyses of covariance (ANCOVA) were conducted for the neonatal body weight data with prenatal treatment and sex as fixed variables and foster mother's treatment as a covariate. Fisher's LSD test was used as a *post hoc* test. Alpha was set to 0.05 in all cases and effect sizes are reported as η_p^2.

Offspring self-administration data were analyzed independently within each reinforcement schedule (FR1, FR2, and FR4) using four-way mixed factorial ANOVAs with sex, maternal treatment, and offspring treatment as between-subjects factors and self-administration day as the within-subjects factor. Offspring self-administration data revealed significant interactions with sex, and thus male and female data were stratified and analyzed independently as separate 3-way ANOVAs. Data from the PR challenge were similarly analyzed separately by sex using separate two-way between-subjects ANOVAs. For the self-administration data, the Fisher's Least Significant Difference (LSD) test was used to further probe differences of statistically significant relationships. For the PR challenge, a Tukey's *post hoc* test was used. For all analyses, significance was defined by a p value <0.05 and effect sizes were reported as η_p^2.

Due to a data storage error, some data were lost from the self-administration datasets. These missing values were interpolated to the mean from the day prior and following. Less than 2% of the total self-administration data was interpolated. Additionally, due to a freezer malfunction, plasma samples from dams from the first cohort (n=12) could not be analyzed. Four dams in the cannabis group and four dams in the vehicle group were excluded for failure to acquire vapor self-administration (<6 vapor deliveries per day on average on last 2 days of acquisition). Outliers were identified as any number above or below 3.29 standard deviations from the mean. Outliers were trimmed to the mean plus or minus 1.0 of the closest value [36]. Outliers comprised less than 1% of the data in this study.

3. Results

3.1. Pregnant rats self-administer cannabis vapor at higher rates and show greater discrimination for the vapor-paired nosepoke

Female rats were assigned to self-administer either cannabis or vehicle vapor from before pregnancy until 24-48 hr prior to birth (see Fig. 1A and 1B for vapor chamber schematic and experimental timeline). There was a large main effect of treatment on vapor deliveries ($F_{(1,26)} = 6.71$, $p = .016$, $\eta_p^2 = .205$; Fig. 1C), with pregnant dams in the cannabis self-administration group receiving more vapor deliveries on average than vehicle dams. There was also a large main effect of day, with vapor deliveries decreasing over the course of pregnancy ($F_{(6.451,167.4)} = 10.1$, $p < .001$, $\eta_p^2 = .28$). There was no interaction of day and treatment ($F_{(20,519)} = 1.15$, $p = .30$, $\eta_p^2 = .042$).

Responding on the active nosepoke decreased significantly over the course of pregnancy ($F_{(7.372,191.7)} = 8.66$, $p < .001$, $\eta_p^2 = .250$; Fig. 1D). There was also a small significant interaction between treatment and day ($F_{(20,520)} = 1.60$, $p = .049$, $\eta_p^2 = .058$) but treatments did not differ significantly on any particular day (all p's >.05). With respect to inactive nosepokes, there was a medium sized interaction between day and treatment ($F_{(20,520)} = 2.23$, $p = .035$, $\eta_p^2 = .092$). Specifically, pregnant dams assigned to self-administer cannabis vapor made fewer inactive nosepokes than vehicle self-administering rats, particularly earlier in gestation ($p < .05$ on days 1, 2, 5, 7 and 8) (data not shown).

There was a large main effect of treatment on nosepoke discrimination ($F_{(1,26)} = 11.92$, $p < .001$, $\eta_p^2 = .314$; Fig. 1E), such that dams that self-administered cannabis showed a greater preference for the active nosepoke than vehicle self-administering dams. Discrimination index did not differ across days ($F_{(10.27,266.9)} = 1.17$, $p = .31$, $\eta_p^2 = .042$), nor was there a significant day x treatment interaction ($F_{(20,520)} = 1.17$, $p = .28$, $\eta_p^2 = .043$). Taken together, these data indicate that pregnant rat dams readily self-administer vaporized cannabis extracts, particularly early in pregnancy, which mirrors cannabis use patterns in pregnant women [1,25].

3.2. Plasma cannabinoid and metabolite concentrations are positively correlated with number of cannabis vapor deliveries

Blood was collected from cannabis self-administering rats on the 10th day of self-administration training. Plasma was then extracted and analyzed for concentrations of Δ^9-THC and its metabolites, 11-OH-THC and THC-COOH. There was a large, positive correlation between number of vapor deliveries received and concentration of Δ^9-THC in the serum, $r(15) = .67$, $p = .003$ (Fig. 2A). There was also a moderate, positive correlation between cannabis vapor deliveries and plasma concentration of 11-OH-THC that did not reach statistical significance, $r(15) = .47$, $p = .059$ (Fig. 2B). Finally, there was a moderate non-significant correlation between number of cannabis vapor deliveries and plasma concentration of THC-COOH, $r(15) = .416$, $p = .097$ (Fig. 2C). Thus, self-administration of cannabis vapor in rat dams produces physiologically relevant concentrations of THC that are positively correlated with the number of vapor deliveries received.

3.3. Maternal cannabis vapor self-administration leads to lower birth weight in exposed offspring

Female rats self-administered cannabis or vehicle vapor prior, during, and after mating until 24-48 hr prior to birth. These experiments were accomplished with three cohorts of female rats for a total of 14 no vapor rats, 16 vehicle rats, and 16 cannabis rats. Of the rats that began self-administration, 9 did not become pregnant or give birth, including 2 in the no vapor group, 2 in the vehicle group, and 5 in the cannabis group. Rats self-administering cannabis had a lower rate of conception (69%) than either the no vapor (86%) or vehicle (88%), though this difference was not statistically significant, X^2 (2, $N = 46$) = 2.144, $p = .34$.

Measurements related to possible consequences of cannabis exposure were taken during pregnancy in a subset of dams. There were no significant differences in total weight gained over the course of pregnancy ($F_{(2,33)} = .031$, $p = .97$, $\eta_p^2 = .002$; Fig. 3A). Average daily food intake differed across days ($F_{(2.108,52.70)} = 16.68$, $p < .001$, $\eta_p^2 = .39$) but there was no main effect of treatment ($F_{(2,25)} = .55$, $p = .58$, $\eta_p^2 = .042$; Fig. 3B) or treatment x day interaction ($F_{(18,225)} = .28$, $p = .99$, $\eta_p^2 = .022$). Additionally, there was no difference in the length of gestation between cannabis and vehicle self-administering dams ($F_{(2,24)} = .42$, $p = .66$; $\eta_p^2 = .034$; Fig. 3C).

On the day following birth, litters were weighed, counted, and sexed. Neither litter size ($F_{(2,34)} = .29$, $p = .75$; $\eta_p^2 = .017$; Fig. 3D) nor litter weight ($F_{(2,25)} = .16$, $p = .85$; $\eta_p^2 = .013$; Fig. 3E) differed by self-administration condition. Furthermore, the percentage of the litter composed of female pups was approximately the same in the cannabis, vehicle, and no vapor groups, X^2 (40, $N = 37$) = 39.93, $p = .47$ (Fig. 3F).

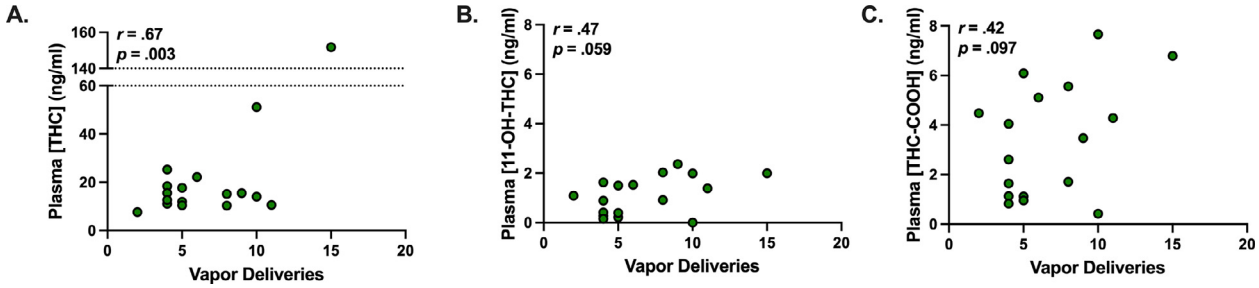

Fig. 2. ***Plasma cannabinoid concentrations are positively correlated with the number of cannabis vapor deliveries earned.*** **(A)** A significant positive correlation was observed between plasma Δ^9-THC concentration at the end of the 60 min session and the number of cannabis vapor deliveries earned during that session. There were also positive correlations between the number of cannabis vapor deliveries earned and plasma concentrations of **(B)** 11-OH-THC and **(C)** THC-COOH metabolites that failed to reach the threshold for statistical significance.

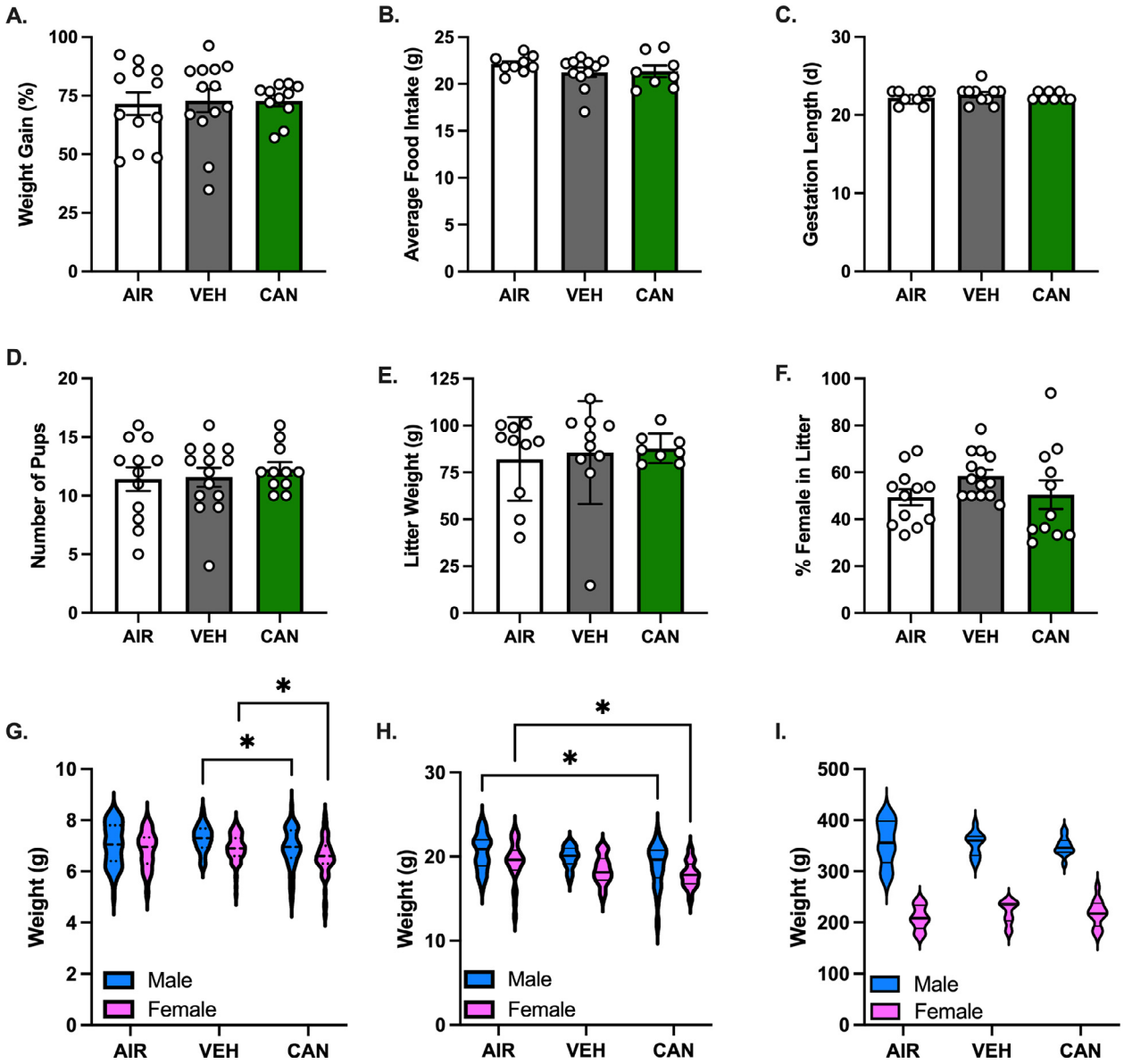

Fig. 3. ***Maternal cannabis vapor self-administration leads to lower birth weight in offspring.*** **(A)** Percent weight gain, **(B)** daily food intake over the course of pregnancy, and **(C)** gestational duration did not differ between dams that self-administered cannabis (CAN), vehicle (VEH), or no vapor (AIR). Analyses of litter-related endpoints revealed no significant group differences with respect to **(D)** litter size, **(E)** litter weight, or **(F)** litter sex ratio. **(G)** The body weights of male and female cannabis-exposed offspring were significantly lower than body weights of vehicle-exposed offspring on postnatal day (P) 1 and **(H)** significantly lower than body weights of no vapor-exposed offspring on P10. **(I)** Body weights of offspring in adulthood were not significantly different between groups. * indicates significant differences between cannabis- and vehicle-exposed groups ($p = .05$).Values indicate mean +/- SEM.

Pups were individually weighed on P1 before cross fostering and again on P10 to assess body weight during the early postnatal period. There was a small sized main effect of treatment ($F_{(2,338)} = 7.87$, $p < .001$, $\eta_p^2 = .048$) and a small sized main effect of sex ($F_{(1,338)} = 16.67$, $p < .001$, $\eta_p^2 = .045$) on P1 weights. Specifically, cannabis-exposed pups weighed less than both no vapor ($p = .044$) and vehicle ($p < .001$; Fig. 3G) pups. There was also a small main effect of treatment ($F_{(2,144)} = 3.54$, $p = .032$, $\eta_p^2 = .049$) and moderate sized effect of sex ($F_{(1,144)} = 14.68$, $p < .001$, $\eta_p^2 = .097$) at P10. At this timepoint, cannabis-exposed pups weighed less than no vapor controls ($p < .001$) but not significantly less than VEH-exposed pups (Fig. 3H).

Offspring were weighed again in young adulthood prior to behavioral testing. There was a large main effect of sex ($F_{(1,63)} = 422.6$, $p < .001$, $\eta_p^2 = .87$), but no effect of treatment ($F_{(2,63)} = .15$, $p = .86$, $\eta_p^2 = .005$) or treatment x sex interaction ($F_{(2,63)} = .55$, $p = .58$, $\eta_p^2 = .017$) (Fig. 3I). Therefore, cannabis-exposed offspring weighed significantly less at birth, but these differences did not continue into adulthood.

3.4. Male cannabis-exposed offspring self-administer less vapor, irrespective of vapor condition

Adult male and female offspring of each prenatal treatment self-administered either cannabis or vehicle vapor over 21 consecutive days according to an escalating schedule of reinforcement (n=8-10/sex/prenatal treatment condition) (Fig. 4A). Offspring self-administration endpoints analyzed revealed significant interactions with sex – thus, male and female data were stratified and analyzed as separate 3-way ANOVAs.

3.4.1. Vapor Deliveries: Male Offspring

In male offspring tested under the FR1 reinforcement schedule, there was a large-sized main effect of prenatal treatment for number of vapor deliveries earned ($F_{(2, 46)} = 4.69$, $p = .014$, $\eta_p^2 = .17$), with cannabis-exposed offspring earning fewer vapor deliveries than VEH-exposed offspring ($p = .014$) and no vapor offspring ($p = .02$) (Fig. 4B). There was no prenatal x postnatal x day interaction ($F_{(20, 460)} = .69$, $p = .84$, $\eta_p^2 = .029$), no prenatal x postnatal interaction ($F_{(2, 46)} = 1.85$, $p = .17$, $\eta_p^2 = .075$), and no prenatal condition x day interaction ($F_{(20, 460)} = 1.20$, $p = .25$, $\eta_p^2 = .049$). However, there was a significant postnatal condition x day interaction ($F_{(10, 460)} = 5.50$, $p < .001$, $\eta_p^2 = .11$), with cannabis self-administering offspring earning more vapor deliveries than vehicle self-administering offspring on days 7 through 10 of FR1 training (all p's $< .024$; Fig. 4B).

When male offspring were advanced to the FR2 reinforcement schedule, there was a large-sized main effect of prenatal treatment for number of vapor deliveries earned ($F_{(2, 46)} = 3.64$, $p = .034$, $\eta_p^2 = .14$; Fig. 4B), with cannabis-exposed offspring earning fewer vapor deliveries than VEH-exposed offspring ($p = .04$) and no vapor offspring ($p = .04$). There was no prenatal x postnatal x day interaction ($F_{(8, 184)} = .41$, $p = .91$, $\eta_p^2 = .018$), no prenatal x postnatal interaction ($F_{(2, 46)} = 1.46$, $p = .24$, $\eta_p^2 = .06$), and no prenatal condition x day interaction ($F_{(8, 184)} = 1.97$, $p = .052$, $\eta_p^2 = .079$). However, there was a significant postnatal condition x day interaction ($F_{(4, 184)} = 2.57$, $p = .04$, $\eta_p^2 = .05$), with cannabis self-administering offspring earning more vapor deliveries than vehicle self-administering offspring on day 1 ($p = .001$) and day 2 ($p = .002$) of FR2 training (Fig. 4B).

Over the five days of FR4 training, there was a large main effect of prenatal condition ($F_{(2, 46)} = 3.85$, $p = .029$, $\eta_p^2 = .14$), with cannabis-exposed male offspring earning fewer vapor deliveries than vehicle-exposed offspring ($p = .02$) but not no vapor offspring ($p = .066$). There was also a large main effect of postnatal condition ($F_{(1, 46)} = 11.74$, $p = .001$, $\eta_p^2 = .20$), with cannabis self-administering rats earning significantly more deliveries than vehicle self-administering rats during FR4 training. There was no main effect of day ($F_{(4, 184)} = 2.08$, $p = .086$, $\eta_p^2 = .04$), no prenatal x postnatal x day interaction ($F_{(8, 184)} = .92$, $p = .50$, $\eta_p^2 = .04$), no prenatal x postnatal interaction ($F_{(2, 46)} = .65$, $p = .53$, $\eta_p^2 = .03$), no prenatal condition x day interaction ($F_{(8, 184)} = 1.97$, $p = .052$, $\eta_p^2 = .079$), and no postnatal condition x day interaction ($F_{(4, 184)} = .63$, $p = .64$, $\eta_p^2 = .01$) (Fig. 4B).

Thus, cannabis self-administering male rats generally earned more vapor reinforcers than vehicle self-administering rats. However, cannabis-exposed male offspring earned fewer vapor deliveries than cannabis-naïve offspring across all three schedules of reinforcement, irrespective of whether were trained to self-administer cannabis or vehicle vapor in adulthood.

3.4.2. Active Nosepokes: Male Offspring

With respect to active nosepokes, there was a large-sized main effect of prenatal condition ($F_{(2, 46)} = 4.03$, $p = .02$, $\eta_p^2 = .15$) but not postnatal condition ($F_{(1, 46)} = .04$, $p = .84$, $\eta_p^2 = .001$). Specifically, cannabis-exposed male offspring made fewer active nosepokes than vehicle-exposed offspring ($p = .02$) and no vapor offspring ($p = .02$). There was no prenatal x postnatal x day interaction ($F_{(20, 460)} = .97$, $p = .50$, $\eta_p^2 = .04$), no prenatal x postnatal interaction ($F_{(2, 46)} = .38$, $p = .68$, $\eta_p^2 = .02$), and no prenatal x day interaction ($F_{(20, 460)} = 1.06$, $p = .39$, $\eta_p^2 = .04$). However, there was a medium-sized postnatal x day interaction ($F_{(10, 460)} = 3.90$, $p < .001$, $\eta_p^2 = .08$), with cannabis self-administering male offspring making fewer active nosepokes than vehicle self-administering offspring on day 1 ($p = .04$), but more active nosepokes than vehicle self-administering offspring on day 9 ($p = .04$) of FR1 training (Fig. 4D).

During FR2 training, there was a medium-sized prenatal condition x day interaction ($F_{(8, 184)} = 2.67$, $p = .009$, $\eta_p^2 = .10$), with cannabis-exposed offspring making fewer active nosepokes than vehicle-exposed offspring on day 1 ($p = .025$), day 4 ($p = .05$), and day 5 ($p = .002$). There was also a significant postnatal condition x day interaction ($F_{(4, 184)} = 2.95$, $p = .022$, $\eta_p^2 = .06$), with cannabis self-administering offspring making more active nosepokes than vehicle self-administering offspring on day 1 ($p = .004$) and day 2 ($p = .012$) of FR2 training (Fig. 4D). There was no prenatal x postnatal x day interaction ($F_{(8, 184)} = .73$, $p = .67$, $\eta_p^2 = .03$) or prenatal x postnatal interaction ($F_{(2, 46)} = .79$, $p = .46$, $\eta_p^2 = .03$).

When male offspring were advanced to the FR4 reinforcement schedule, there was a large-sized main effect of postnatal condition ($F_{(1, 46)} = 9.64$, $p = .003$, $\eta_p^2 = .17$), with cannabis self-administering offspring making more active nosepokes than vehicle self-administering offspring. There was also a medium-sized prenatal condition x day interaction ($F_{(8, 184)} = 2.55$, $p = .012$, $\eta_p^2 = .10$), with cannabis-exposed male offspring making fewer active nosepokes than vehicle-exposed offspring on day 1 ($p = .045$) and day 2 ($p = .021$), and fewer active nosepokes than no vapor offspring on day 3 ($p = .04$). There was no prenatal x postnatal x day interaction ($F_{(8, 184)} = 1.04$, $p = .41$, $\eta_p^2 = .04$), no prenatal x postnatal condition interaction ($F_{(2, 46)} = .34$, $p = .72$, $\eta_p^2 = .01$), and no postnatal condition x day interaction ($F_{(4, 184)} = .66$, $p = .62$, $\eta_p^2 = .01$).

3.4.3. Inactive Nosepokes: Male Offspring

Responses made on the inactive nosepoke did not differ by prenatal condition ($F_{(2, 46)} = .18$, $p = .84$, $\eta_p^2 = .008$) or postnatal condition ($F_{(1, 46)} = 3.99$, $p = .052$, $\eta_p^2 = .08$), but there was a large main effect of day ($F_{(10, 460)} = 11.70$, $p < .001$, $\eta_p^2 = .20$), with male offspring making fewer inactive nosepokes over the course of FR1 training (Fig. 4F). Additionally, there was no prenatal x postnatal x day interaction ($F_{(20, 460)} = .52$, $p = .96$, $\eta_p^2 = .02$), no prenatal x postnatal condition interaction ($F_{(2, 46)} = .10$, $p = .90$, $\eta_p^2 = .004$), no prenatal condition x day interaction ($F_{(20, 460)} = .44$, $p = .98$, $\eta_p^2 = .02$), and no postnatal condition x day interaction ($F_{(10, 460)} = 1.80$, $p = .06$, $\eta_p^2 = .04$).

During FR2 training, there was no main effect of prenatal condition ($F_{(2, 46)} = .64$, $p = .53$, $\eta_p^2 = .03$), postnatal condition ($F_{(1, 46)} = 2.19$, $p = .15$, $\eta_p^2 = .05$), or day ($F_{(4, 184)} = .60$, $p = .67$, $\eta_p^2 = .01$) on the

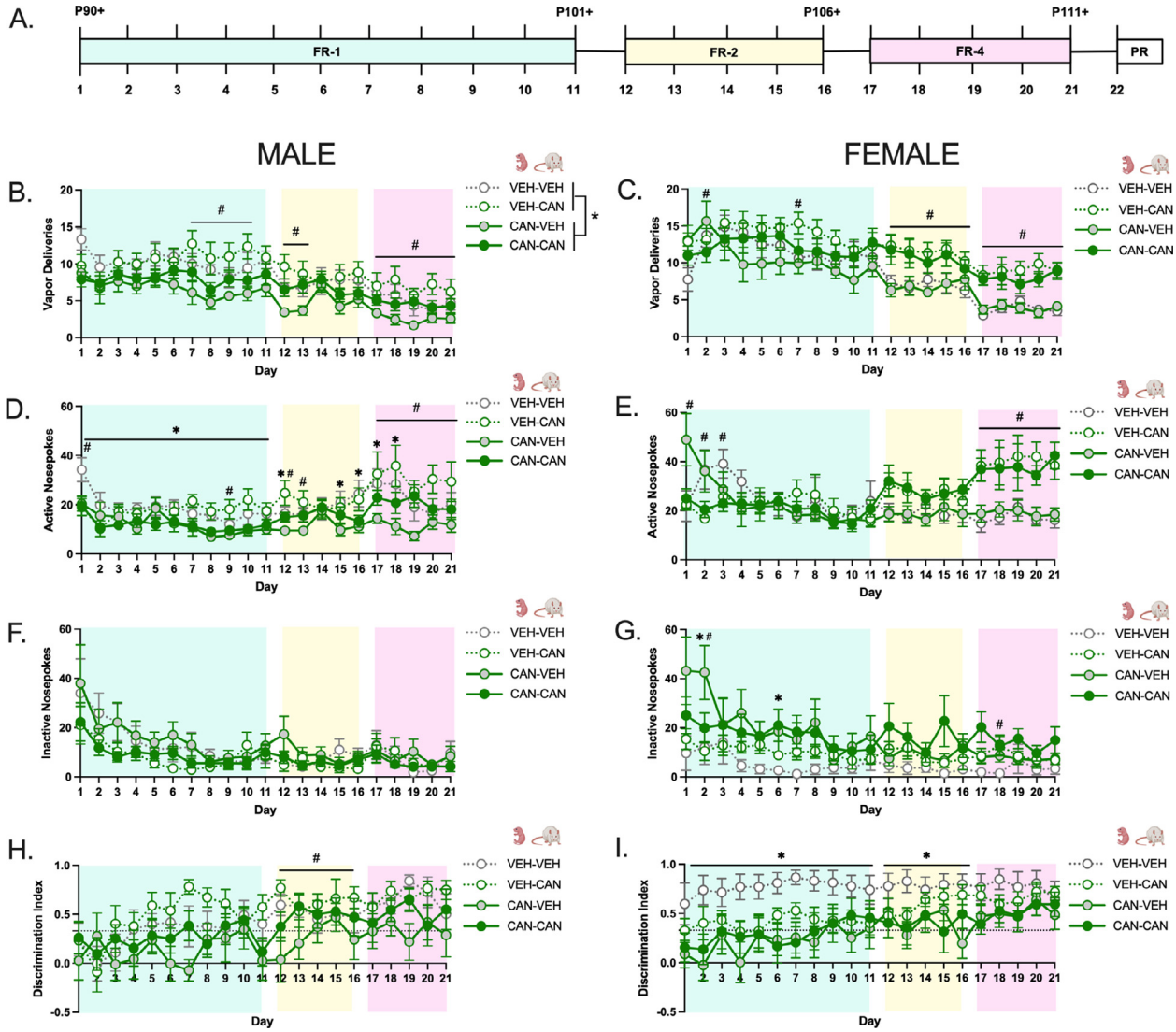

Fig. 4. ***Male cannabis-exposed offspring self-administer less vapor, irrespective of vapor condition.*** **(A)** Timeline of self-administration. **(B)** Cannabis-exposed male offspring earned fewer vapor deliveries than vehicle-exposed offspring across each reinforcement schedule. Cannabis self-administering rats earned more vapor deliveries than vehicle self-administering rats on days 7-10 of FR1 training, days 1 and 2 of FR2 training, and days 1-5 of FR4 training. **(C)** Female cannabis self-administering offspring earned fewer vapor deliveries than vehicle self-administering offspring on day 2 of FR1 training but earned more vapor deliveries than vehicle self-administering offspring on day 7 and across all days of FR2 and FR4 training. Prenatal cannabis exposure did not affect the number of vapor deliveries earned by female offspring when tested in adulthood. **(D)** Male cannabis-exposed offspring made fewer active nosepokes than vehicle-exposed offspring across the duration of FR1 training, on days 1, 4, and 5 of FR2 training, and on the first two days of FR4 training. Cannabis self-administering male offspring made fewer active nosepokes than vehicle self-administering offspring on day 1 of FR1 training and days 1 and 2 of FR4 training, but more active nosepokes than vehicle self-administering offspring on day 9 of FR1 training and days 1 and 2 of FR2 training. **(E)** Prenatal cannabis exposure did not affect the number of active nosepokes made by female offspring in adulthood. Female cannabis self-administering offspring made fewer active nosepokes than vehicle self-administering offspring on the first three days of FR1 training but made more active nosepokes than vehicle self-administering offspring across the entire FR4 training period. **(F)** Inactive nosepokes were similar across prenatal and postnatal conditions at each reinforcement schedule in male offspring but declined over the course of FR1 and FR4 training. **(G)** Cannabis-exposed female offspring made more inactive nosepokes than vehicle-exposed offspring on day 2 and day 6 of FR1 training. Cannabis self-administering female offspring made fewer inactive nosepokes than vehicle self-administering offspring on day 2 of FR1 training. Vehicle-exposed female offspring that self-administered cannabis vapor in adulthood made more inactive responses than vehicle-exposed offspring trained to self-administer vehicle vapor, specifically on day 2 of FR4 training. **(H)** The daily discrimination index did not differ according to prenatal treatment in male offspring. Cannabis self-administering male rats showed better discrimination for the active nosepoke relative to vehicle self-administering rats during FR2 training. **(I)** The daily discrimination index in female offspring differed according to prenatal condition, with cannabis-exposed female offspring exhibiting worse discrimination than vehicle-exposed offspring over the course of FR1 and FR2 reinforcement schedules. VEH = vehicle, CAN = cannabis. Newborn rat symbols (left) and adult rat symbols (right) are provided above Fig. legends to indicate prenatal treatment condition and assigned vapor condition in adulthood, respectively. * indicates a significant difference between offspring prenatally exposed to cannabis or vehicle vapor ($p \leq .05$). # indicates a significant difference between offspring that self-administered cannabis vs. vehicle vapor in adulthood, irrespective of prenatal history ($p \leq .05$). Air-exposed offspring did not differ from VEH-exposed offspring on any self-administration endpoint. Thus, for clarity, only rats prenatally exposed to cannabis and vehicle are shown on graphs. Values indicate mean +/- SEM. n=8-10/sex/prenatal treatment condition.

number of inactive nosepokes made by male offspring. There was also no prenatal x postnatal x day interaction ($F_{(8, 184)} = 1.34$, $p = .22$, $\eta_p^2 = .06$), no prenatal x postnatal condition interaction ($F_{(2, 46)} = .24$, $p = .79$, $\eta_p^2 = .01$), no prenatal x day interaction ($F_{(8, 184)} = 1.91$, $p = .06$, $\eta_p^2 = .08$), and no postnatal x day interaction ($F_{(4, 184)} = .57$, $p = .68$, $\eta_p^2 = .01$) (Fig. 4F).

During FR4 training, there was no main effect of prenatal condition ($F_{(2, 46)} = .06$, $p = .94$, $\eta_p^2 = .003$) or postnatal condition ($F_{(1, 46)} = .06$, $p = .82$, $\eta_p^2 = .001$), but there was a main effect of day ($F_{(4, 184)} = 3.29$, $p = .01$, $\eta_p^2 = .07$), such that the number of inactive nosepokes made by male offspring significantly decreased over the course of FR4 training. There was no prenatal x postnatal x day interaction ($F_{(8, 184)} = 1.06$, $p = .39$, $\eta_p^2 = .04$), no prenatal x postnatal condition interaction ($F_{(2, 46)} = 1.68$, $p = .20$, $\eta_p^2 = .07$), no prenatal condition x day interaction ($F_{(8, 184)} = .85$, $p = .56$, $\eta_p^2 = .04$), and no postnatal condition x day interaction ($F_{(4, 184)} = .25$, $p = .91$, $\eta_p^2 = .01$) (Fig. 4F).

3.4.4. Discrimination Index: Male Offspring

The discrimination index in male rats did not differ according to prenatal condition ($F_{(2, 46)} = 1.70$, $p = .19$, $\eta_p^2 = .07$) or postnatal condition ($F_{(1, 46)} = 2.07$, $p = .16$, $\eta_p^2 = .04$), but there was a medium-sized effect of day ($F_{(10, 460)} = 4.50$, $p < .001$, $\eta_p^2 = .09$), with offspring generally showing better discrimination over the course of FR1 training (Fig. 4H). There was no prenatal x postnatal x day interaction ($F_{(20, 460)} = .62$, $p = .90$, $\eta_p^2 = .03$), no prenatal x postnatal interaction ($F_{(2, 46)} = .002$, $p = .998$, $\eta_p^2 < .001$), no prenatal x day interaction ($F_{(20, 460)} = 1.51$, $p = .073$, $\eta_p^2 = .06$), and no postnatal x day interaction ($F_{(10, 460)} = 1.08$, $p = .38$, $\eta_p^2 = .02$).

On the FR2 reinforcement schedule, there was no effect of prenatal condition ($F_{(2, 46)} = 2.40$, $p = .10$, $\eta_p^2 = .09$) or day ($F_{(4, 184)} = .87$, $p = .49$, $\eta_p^2 = .02$), but there was a main effect of postnatal condition ($F_{(1, 46)} = 4.67$, $p = .04$, $\eta_p^2 = .09$) with cannabis self-administering male rats showing better discrimination for the active nosepoke relative to vehicle self-administering rats (Fig. 4H). There was no prenatal x postnatal x day interaction ($F_{(8, 184)} = .47$, $p = .88$, $\eta_p^2 = .02$), no postnatal condition x day interaction ($F_{(4, 184)} = .22$, $p = .93$, $\eta_p^2 = .005$), no prenatal condition x day interaction ($F_{(8, 184)} = 1.16$, $p = .33$, $\eta_p^2 = .05$), and no prenatal x postnatal interaction ($F_{(2, 46)} = .11$, $p = .90$, $\eta_p^2 = .005$).

Finally, on the FR4 reinforcement schedule, there was no main effect of prenatal condition ($F_{(2, 46)} = 2.56$, $p = .09$, $\eta_p^2 = .10$), postnatal condition ($F_{(1, 46)} = .42$, $p = .52$, $\eta_p^2 = .009$), or day ($F_{(4, 184)} = 1.09$, $p = .36$, $\eta_p^2 = .02$). There was no prenatal x postnatal x day interaction ($F_{(8, 184)} = 1.14$, $p = .34$, $\eta_p^2 = .05$), no postnatal condition x day interaction ($F_{(4, 184)} = .24$, $p = .91$, $\eta_p^2 = .005$), no prenatal condition x day interaction ($F_{(8, 184)} = .33$, $p = .95$, $\eta_p^2 = .01$), and no prenatal x postnatal interaction ($F_{(2, 46)} = .57$, $p = .57$, $\eta_p^2 = .02$) (Fig. 4H).

3.5. Female offspring self-administer more cannabis vapor than vehicle vapor under more demanding reinforcement schedules, irrespective of prenatal history

3.5.1. Vapor Deliveries: Female Offspring

In female offspring tested under the FR1 reinforcement schedule, there were no main effects of prenatal condition ($F_{(2, 48)} = 1.20$, $p = .31$, $\eta_p^2 = .048$) or postnatal condition ($F_{(1, 48)} = .46$, $p = .50$, $\eta_p^2 = .01$) on the number of vapor deliveries earned. There was no prenatal x postnatal x day interaction ($F_{(20, 480)} = .99$, $p = .47$, $\eta_p^2 = .04$) or prenatal x day interaction ($F_{(20, 480)} = .97$, $p = .50$, $\eta_p^2 = .04$), but there was a significant postnatal x day interaction ($F_{(10, 480)} = 2.72$, $p = .003$, $\eta_p^2 = .054$), with cannabis self-administering female offspring receiving fewer vapor deliveries than vehicle self-administering offspring on day 2 ($p = .023$), but more vapor deliveries than vehicle self-administering offspring on day 7 ($p = .023$) of FR1 training (Fig. 4C).

When female offspring were advanced to the FR2 reinforcement schedule, there was no effect of prenatal treatment ($F_{(2, 48)} = .78$, $p = .46$, $\eta_p^2 = .03$), but a large-sized main effect of postnatal treatment for number of vapor deliveries earned ($F_{(1, 48)} = 9.21$, $p = .034$, $\eta_p^2 = .14$), with cannabis-self-administering offspring earning more vapor deliveries than vehicle self-administering offspring (Fig. 4C). There was also a main effect of day ($F_{(4, 192)} = 2.45$, $p = .048$, $\eta_p^2 = .05$), such that the number of vapor deliveries earned significantly decreased over the course of FR2 training. There was no prenatal x postnatal x day interaction ($F_{(8, 192)} = .78$, $p = .62$, $\eta_p^2 = .03$), no prenatal x postnatal interaction ($F_{(2, 48)} = 1.39$, $p = .26$, $\eta_p^2 = .06$), no prenatal condition x day interaction ($F_{(8, 192)} = .66$, $p = .73$, $\eta_p^2 = .03$), and no postnatal x day interaction ($F_{(4, 192)} = .80$, $p = .53$, $\eta_p^2 = .02$).

Over the five days of FR4 training, there was no effect of prenatal condition ($F_{(2, 48)} = 3.07$, $p = .056$, $\eta_p^2 = .11$), but there was large-sized main effect of postnatal condition ($F_{(1, 48)} = 20$, $p < .001$, $\eta_p^2 = .29$), with cannabis self-administering female rats earning significantly more vapor deliveries than vehicle self-administering rats (Fig. 4C). There was no main effect of day ($F_{(4, 192)} = 1.50$, $p = .21$, $\eta_p^2 = .03$), no prenatal x postnatal x day interaction ($F_{(8, 192)} = .92$, $p = .50$, $\eta_p^2 = .04$), no prenatal condition x day interaction ($F_{(8, 192)} = 1.78$, $p = .08$, $\eta_p^2 = .07$), no postnatal condition x day interaction ($F_{(4, 192)} = .47$, $p = .76$, $\eta_p^2 = .01$), and no prenatal x postnatal condition interaction ($F_{(2, 48)} = 3.12$, $p = .053$, $\eta_p^2 = .12$).

These data collectively corroborate recent studies from our laboratory (Glodosky et al., 2020), demonstrating that cannabis vapor supports robust responding in female rats, particularly under more demanding schedules of reinforcement. However, responding for cannabis vapor in adulthood is unaffected by prenatal cannabis exposure in female rats.

3.5.2. Active Nosepokes: Female Offspring

With respect to active nosepokes, there was no main effect of prenatal condition ($F(2, 48) = .31$, $p = .73$, $\eta_p^2 = .01$), but there was a medium-sized postnatal x day interaction ($F_{(10, 480)} = 3.61$, $p < .001$, $\eta_p^2 = .07$), with cannabis self-administering female offspring making fewer active nosepokes than vehicle self-administering offspring on day 1 ($p = .01$), day 2 ($p < .001$), and day 3 ($p = .01$) (Fig. 4E). There was no prenatal x postnatal x day interaction ($F_{(20, 480)} = 1.10$, $p = .35$, $\eta_p^2 = .04$), no prenatal x postnatal interaction ($F_{(2, 48)} = 1.06$, $p = .35$, $\eta_p^2 = .04$), and no prenatal x day interaction ($F_{(20, 480)} = 1.39$, $p = .12$, $\eta_p^2 = .05$).

During FR2 training, there were no main effects of prenatal condition ($F_{(2, 48)} = 1.12$, $p = .33$, $\eta_p^2 = .05$), postnatal condition ($F_{(1, 48)} = .89$, $p = .35$, $\eta_p^2 = .02$), or day ($F_{(4, 192)} = 1.60$, $p = .18$, $\eta_p^2 = .03$) with respect to active nosepokes in female offspring. There was also no significant prenatal x postnatal x day interaction ($F_{(8, 192)} = .69$, $p = .70$, $\eta_p^2 = .03$), no prenatal x postnatal condition interaction ($F_{(2, 48)} = 1.86$, $p = .17$, $\eta_p^2 = .07$), no prenatal condition x day interaction ($F_{(8, 192)} = .47$, $p = .87$, $\eta_p^2 = .02$), and no postnatal condition x day interaction ($F_{(4, 192)} = .46$, $p = .77$, $\eta_p^2 = .01$) (Fig. 4E).

When female offspring were advanced to the FR4 reinforcement schedule, there was a large-sized main effect of postnatal condition ($F_{(1, 48)} = 7.65$, $p = .008$, $\eta_p^2 = .14$), with female cannabis self-administering rats making more active nosepokes than vehicle self-administering rats (Fig. 4E). There was no main effect of prenatal condition ($F_{(2, 48)} = 1.17$, $p = .32$, $\eta_p^2 = .05$) or day ($F_{(4, 192)} = 1.30$, $p = .27$, $\eta_p^2 = .03$). There was also no prenatal x postnatal x day interaction ($F_{(8, 192)} = .59$, $p = .79$, $\eta_p^2 = .02$), no prenatal x postnatal condition interaction ($F_{(2, 48)} = 2.64$, $p = .08$, $\eta_p^2 = .10$), no prenatal condition x day interaction ($F_{(8, 192)} = 1.07$, $p = .38$, $\eta_p^2 = .04$), and no postnatal condition x day interaction ($F_{(4, 192)} = .35$, $p = .84$, $\eta_p^2 = .01$).

3.5.3. Inactive Nosepokes: Female Offspring

With respect to inactive nosepokes, there was no prenatal x postnatal x day interaction ($F_{(20, 480)} = .72$, $p = .81$, $\eta_p^2 = .03$) or prenatal x postnatal interaction ($F_{(2, 48)} = 1.36$, $p = .27$, $\eta_p^2 = .05$), but there was a medium-sized prenatal x day interaction ($F_{(20, 480)} = 1.76$, $p = .02$, $\eta_p^2 = .07$). The interaction was probed using one-way ANOVAs examining the effect of prenatal exposure at each day of FR1 training. Significant main effects were observed on days 1, 2, and 6. Post-hoc analyses revealed that no vapor offspring made more inactive nosepokes than vehicle-exposed offspring on day 1 ($p = .008$) and day 2 ($p = .02$) (data not shown). Cannabis-exposed offspring also made more inactive nosepokes than vehicle-exposed offspring on day 2 ($p = .03$) and day 6 ($p = .01$) (Fig. 4G). There was also a significant postnatal x day interaction ($F_{(10, 480)} = 2.82$, $p = .002$, $\eta_p^2 = .06$), with cannabis self-administering rats making fewer inactive nosepokes than vehicle self-administering rats on day 2 ($p = .01$) (Fig. 4G).

During FR2 training, there was no main effect of prenatal condition ($F_{(2, 48)} = 2.36$, $p = .11$, $\eta_p^2 = .09$), postnatal condition ($F_{(1, 48)} = .47$, $p = .50$, $\eta_p^2 = .01$), or day ($F_{(4, 192)} = 1.79$, $p = .13$, $\eta_p^2 = .04$) on the number of inactive nosepokes made by female offspring. There was also no prenatal x postnatal x day interaction ($F_{(8, 192)} = 1.71$, $p = .10$, $\eta_p^2 = .07$), no prenatal x postnatal condition interaction ($F_{(2, 48)} = 2.54$, $p = .09$, $\eta_p^2 = .10$), no prenatal condition x day interaction ($F_{(8, 192)} = .70$, $p = .69$, $\eta_p^2 = .03$), and no postnatal condition x day interaction ($F_{(4, 192)} = .69$, $p = .60$, $\eta_p^2 = .01$) (Fig. 4G).

During FR4 training, there was a significant prenatal x postnatal x day interaction ($F_{(8, 192)} = 2.11$, $p = .036$, $\eta_p^2 = .08$). Post-hoc analyses examining differences in inactive nosepokes across FR4 training days according to each prenatal condition revealed that vehicle-exposed female offspring trained to self-administer cannabis vapor in adulthood made more inactive responses than vehicle-exposed offspring trained to self-administer vehicle vapor on day 2 of FR4 training ($p = .02$). No other significant group differences in inactive nosepokes were observed in female offspring under this reinforcement schedule (all p's $> .05$) (Fig. 4G).

3.5.4. Discrimination Index: Female Offspring

There was a large-sized main effect of prenatal condition on discrimination index in female offspring ($F_{(2, 48)} = 4.71$, $p = .014$, $\eta_p^2 = .16$), with cannabis-exposed offspring exhibiting worse discrimination than vehicle-exposed offspring ($p = .008$), but not no vapor offspring ($p = .70$) during FR1 training. There was also a medium-sized effect of day ($F_{(10, 480)} = 3.61$ $p < .001$, $\eta_p^2 = .07$), with female offspring generally showing better discrimination over the course of FR1 training (Fig. 4I). There was no prenatal x postnatal x day interaction ($F_{(20, 480)} = .64$, $p = .88$, $\eta_p^2 = .03$), no prenatal x postnatal interaction ($F_{(2, 48)} = 2.85$, $p = .068$, $\eta_p^2 = .11$), no prenatal x day interaction ($F_{(20, 480)} = .90$, $p = .58$, $\eta_p^2 = .04$), and no postnatal x day interaction ($F_{(10, 480)} = .60$, $p = .82$, $\eta_p^2 = .01$).

When female rats advanced to the FR2 reinforcement schedule, there was no main effect of postnatal condition ($F_{(1, 48)} = .04$ $p = .85$, $\eta_p^2 = .001$) or day ($F_{(4, 192)} = 1.93$ $p = .11$, $\eta_p^2 = .04$), but there was a large-sized main effect of prenatal condition ($F_{(2, 48)} = 3.95$, $p = .03$, $\eta_p^2 = .14$), with cannabis-exposed rats exhibiting worse discrimination for the active nosepoke relative to vehicle-exposed rats ($p = .009$) but not relative to no vapor rats ($p = .41$) (Fig. 4I). There was no prenatal x postnatal x day interaction ($F_{(8, 192)} = 1.52$, $p = .15$, $\eta_p^2 = .06$), no postnatal condition x day interaction ($F_{(4, 192)} = .74$, $p = .56$, $\eta_p^2 = .02$), no prenatal condition x day interaction ($F_{(8, 192)} = 1.05$, $p = .40$, $\eta_p^2 = .04$), and no prenatal x postnatal interaction ($F_{(2, 48)} = 2.21$, $p = .12$, $\eta_p^2 = .08$).

Finally, on the FR4 reinforcement schedule, there was no main effect of prenatal condition ($F_{(2, 48)} = 2.51$, $p = .09$, $\eta_p^2 = .09$), postnatal condition ($F_{(1, 48)} = .02$, $p = .88$, $\eta_p^2 < .001$), or day ($F_{(4, 192)} = 1.12$, $p = .35$, $\eta_p^2 = .02$). There was no prenatal x postnatal x day interaction ($F_{(8, 192)} = 1.28$, $p = .26$, $\eta_p^2 = .05$), no postnatal condition x day interaction ($F_{(4, 192)} = 1.66$, $p = .16$, $\eta_p^2 = .03$), no prenatal condition x day interaction ($F_{(8, 192)} = .31$, $p = .96$, $\eta_p^2 = .01$), and no prenatal x postnatal interaction ($F_{(2, 48)} = .71$, $p = .50$, $\eta_p^2 = .03$) (Fig. 4I).

3.6. Cannabis-exposed offspring do not exhibit greater motivation for cannabis vapor during a progressive ratio challenge

On the last day of self-administration, rats underwent a PR challenge. In male rats, PR breakpoint did not differ according to prenatal vapor condition ($F_{(2,46)} = 2.76$, $p = .074$, $\eta_p^2 = .11$) or postnatal vapor condition ($F_{(1,46)} = 2.28$, $p = .14$, $\eta_p^2 = .047$), nor were there any prenatal x postnatal group interactions ($F_{(2,46)} = .88$, $p = .42$, $\eta_p^2 = .037$) (Fig. 5A). Similarly, total vapor deliveries earned in males were similar across prenatal vapor conditions ($F_{(2,46)} = 2.07$, $p = .14$, $\eta_p^2 = .083$) and although there was a medium-sized effect of postnatal vapor condition, this was not statistically significant ($F_{(1,46)} = 3.20$, $p = .08$, $\eta_p^2 = .065$). There was also no prenatal x postnatal group interaction for total vapor deliveries earned ($F_{(2,46)} = .017$, $p = .98$, $\eta_p^2 < .001$) (Fig. 5B).

With respect to active nosepokes, there was no significant effect of prenatal vapor condition ($F_{(2,46)} = 1.17$, $p = .32$, $\eta_p^2 = .048$) or postnatal vapor condition ($F_{(1,46)} = 3.52$, $p = .067$, $\eta_p^2 = .071$). There was also no prenatal x postnatal group interaction for number of active nosepokes ($F_{(2,46)} = .078$, $p = .93$, $\eta_p^2 = .003$) (Fig. 5C). The number of inactive nosepokes also did not differ according to prenatal vapor condition ($F_{(2,46)} = 2.12$, $p = .13$, $\eta_p^2 = .084$) or postnatal vapor condition ($F_{(1,46)} = .11$, $p = .74$, $\eta_p^2 = .002$), nor were there any prenatal x postnatal group interactions ($F_{(2,46)} = 1.01$, $p = .37$, $\eta_p^2 = .042$) (data not shown). However, there was a large main effect of prenatal treatment on discrimination index during the PR challenge ($F_{(2,46)} = 4.02$, $p = .025$, $\eta_p^2 = .149$). Regardless of their assigned vapor, cannabis-exposed male offspring showed poorer discrimination for the vapor-paired nosepoke compared to vehicle-exposed males ($p < .05$; Fig. 5D). There were no main effects of postnatal vapor condition on discrimination index ($F_{(1,46)} = .036$, $p = .85$, $\eta_p^2 < .001$), nor was there an interaction between prenatal condition and postnatal condition ($F_{(2,46)} = .82$, $p = .45$, $\eta_p^2 = .034$). Thus, the PR data agree with the self-administration data above and suggest that the reinforcing properties of vapor are blunted in male rats prenatally exposed to cannabis.

In female rats, PR breakpoint did not differ according to prenatal vapor condition ($F_{(2,48)} = 2.07$, $p = .14$, $\eta_p^2 = .079$). However, there was a medium-sized effect of postnatal vapor condition ($F_{(1,48)} = 5.28$, $p = .026$, $\eta_p^2 = .10$), with female rats exhibiting higher breakpoints for cannabis vapor relative to vehicle vapor (Fig. 5E). There was no prenatal x postnatal group interaction ($F_{(2,48)} = .36$, $p = .70$, $\eta_p^2 = .015$), suggesting that higher breakpoints for cannabis vapor were achieved in females, irrespective of prenatal history. Similarly, there was no effect of prenatal vapor condition on total vapor deliveries earned ($F_{(2,48)} = 1.40$, $p = .26$, $\eta_p^2 = .055$), though there was a large-sized effect of postnatal vapor condition ($F_{(1,48)} = 17.11$, $p < .0001$, $\eta_p^2 = .263$), with female rats earning more cannabis vapor deliveries than vehicle vapor deliveries during the PR session (Fig. 5F). There was no significant prenatal x postnatal group interaction for total vapor deliveries earned ($F_{(2,48)} = 1.71$, $p = .19$, $\eta_p^2 = .067$).

There was no effect of prenatal vapor condition on the number of active nosepokes ($F_{(2,48)} = 1.57$, $p = .22$, $\eta_p^2 = .061$), though there was a medium-sized effect of postnatal vapor condition ($F_{(1,48)} = 6.73$, $p < .013$, $\eta_p^2 = .123$), with female rats making more active nosepokes for cannabis vapor than vehicle vapor during the PR session (Fig. 5G). There was no significant prenatal x postnatal group interaction for total active nosepokes ($F_{(2,48)} = 1.14$, $p = .33$, $\eta_p^2 = .045$), suggesting that the difference in active responding for cannabis vapor was irrespective of prenatal history. With respect to inactive nosepokes, there was no significant effect of prenatal vapor condition ($F_{(2,48)} = 1.98$, $p = .15$,

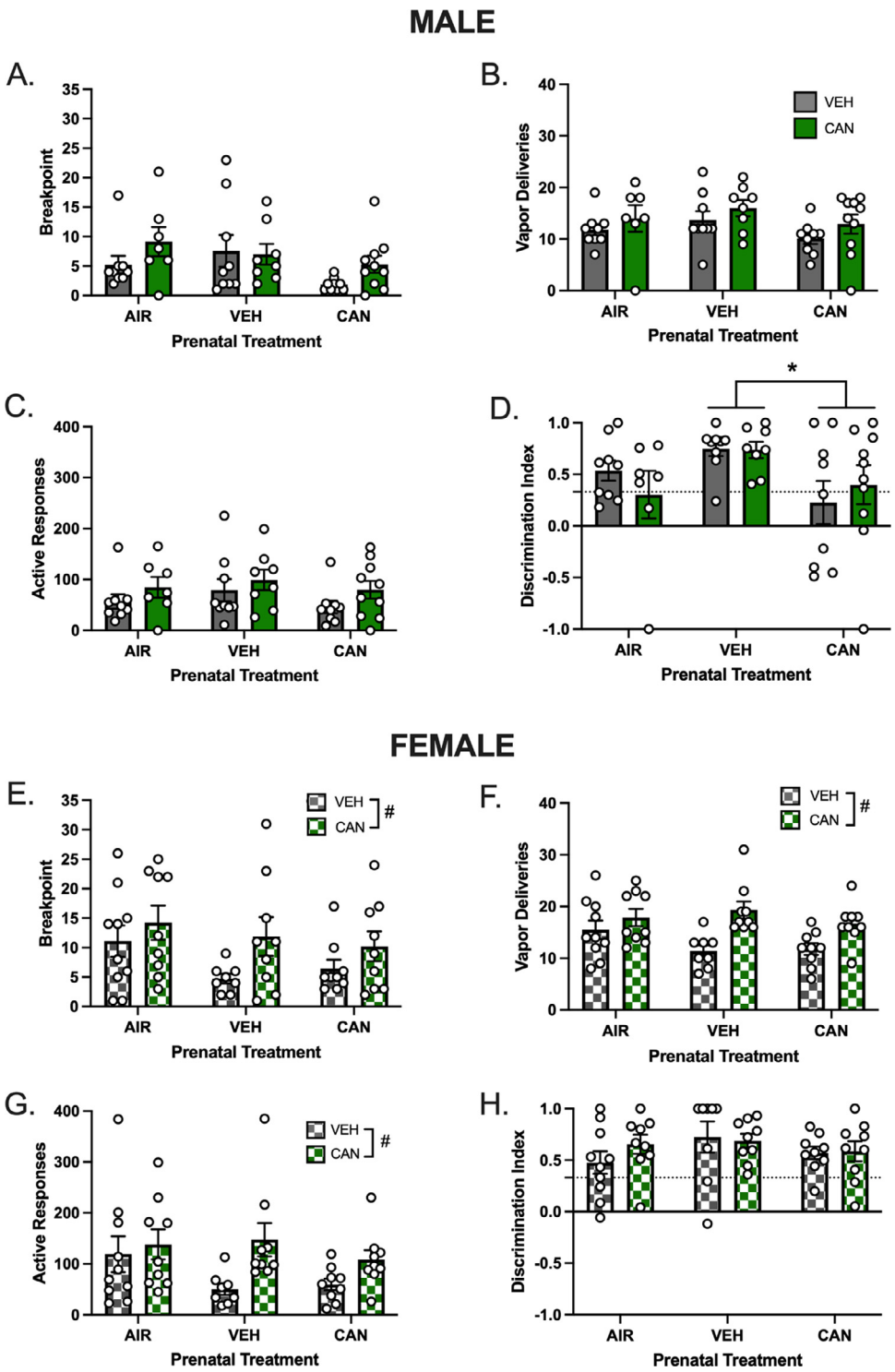

Fig. 5. ***Cannabis-exposed offspring do not exhibit greater motivation for cannabis vapor during a progressive ratio challenge.*** Male offspring in the cannabis (CAN) condition did not differ from male offspring in the vehicle (VEH) or no vapor (AIR) condition on **(A)** PR breakpoint, **(B)** the number of vapor deliveries earned, or **(C)** the number of active nosepokes made. **(D)** Male cannabis-exposed offspring displayed significantly worse discrimination for the active nosepoke compared to vehicle-exposed offspring. Female cannabis-exposed offspring did not differ from vehicle or no vapor-exposed offspring on **(E)** breakpoint, **(F)** vapor deliveries earned, **(G)** active nosepokes, or **(H)** discrimination index. However, female offspring assigned to self-administer cannabis vapor in adulthood showed significantly higher breakpoints, received significantly more vapor deliveries, and made significantly more active nosepokes than female offspring assigned to self-administer vehicle vapor, irrespective of prenatal history. * indicates a significant difference between offspring prenatally exposed to cannabis or vehicle vapor ($p \leq .05$). # indicates a significant difference between offspring that self-administered cannabis vs. vehicle vapor in adulthood, irrespective of prenatal history ($p \leq .05$). Dotted horizontal lines in **(C)** and **(F)** represent 2:1 active:inactive response ratio. Values indicate mean +/- SEM. n=8-10/sex/prenatal treatment condition.

$\eta_p^2 = .076$), postnatal vapor condition ($F_{(1,48)} = .83$, $p = .37$, $\eta_p^2 = .017$), and no significant prenatal x postnatal group interaction ($F_{(2,48)} = 2.28$, $p = .11$, $\eta_p^2 = .087$) (data not shown). Finally, with respect to discrimination index, there was no significant effect of prenatal vapor condition ($F_{(2,48)} = 1.18$, $p = .32$, $\eta_p^2 = .047$), postnatal vapor condition ($F_{(1,48)} = .42$, $p = .52$, $\eta_p^2 = .009$), and no significant prenatal x postnatal group interaction ($F_{(2,48)} = .64$, $p = .53$, $\eta_p^2 = .026$) (Fig. 5H).

These data collectively indicate that cannabis vapor self-administration in pregnant rat dams does not augment the reinforcing properties of cannabis vapor in their male or female offspring when tested in adulthood, contrary to observations in human studies [9,12,27,34].

4. Discussion

The goals of the current study were twofold. First, we investigated whether female rats would self-administer cannabis vapor throughout pregnancy, thereby establishing a more translational model for maternal cannabis use. We found that pregnant rat dams self-administered cannabis vapor at relatively stable rates throughout pregnancy, with

a decline in active responding and the number of daily vapor deliveries earned towards the end of gestation. Importantly, we observed significantly lower birthweights in neonatal rats exposed to cannabis, which mirrors one of the most commonly observed effects of maternal cannabis use in human studies [16]. Second, we used this model to examine whether prenatal cannabis exposure impacts rates of cannabis self-administration in offspring. Effects differed by sex such that male cannabis-exposed offspring made fewer active responses and earned fewer vapor deliveries than vehicle-exposed offspring, regardless of their assigned vapor condition. Conversely, female offspring showed higher rates of responding for cannabis relative to vehicle, which is in line with previous studies from our lab [15] – however, rates of vapor self-administration were unaffected by prenatal cannabis exposure. Altogether, these data demonstrate feasibility of response-contingent cannabis vapor delivery in pregnant rat dams and further indicate paradoxical suppressive effects on vapor self-administration in adult male offspring that is discordant with extant clinical and preclinical literature.

Our data indicate that in rat dams, 1) cannabis vapor elicits more active responding than vehicle vapor, 2) dams will self-administer cannabis to reach physiologically relevant circulating concentrations of THC, and 3) responding for vapor declines as gestation progresses. To our knowledge, this is the first study to examine self-administration of cannabinoids during pregnancy in rats. Other lab groups have, however, used self-administration with other drugs to model maternal use during pregnancy [18,28,30,33,39]. In their study of prenatal effects of maternal nicotine use, LeSage et al. (2007) found that female rats self-administered less nicotine during the last week of pregnancy compared to baseline. However, other preclinical studies typically do not show a decline in responding towards the end of pregnancy in self-administration of methamphetamine [30], alcohol [28], oxycodone [39], and caffeine [33], which is in direct contrast with our data. It is possible that the lack of decline may be due to relative differences in the reinforcing properties of these drugs compared to cannabis, which generally has a lower abuse liability [50]. It is worth noting however that this pattern of responding is consistent with human studies indicating significant reductions in cannabis use over the course of pregnancy [1,25]. Moreover, there may be bioaccumulation of THC and its primary metabolites in the fetus and placenta due to the lipophilic nature of cannabinoids [4], resulting in greater availability of cannabinoids during times with less maternal intake.

Our data further revealed that the number of cannabis vapor deliveries earned during a self-administration session prior to conception was significantly correlated with plasma THC (but not metabolite) concentrations. This is in line with observations from our prior studies [14], which together suggest that higher rates of responding are associated with higher plasma THC concentrations. However, like in that study, concentrations of THC were somewhat lower than what is typically observed following cannabis consumption in humans [52] or non-contingent vapor delivery in rodents [3,4]. This is likely because rats self-administering cannabis vapor display a loading dose phenomenon whereby most responding occurs during the first 15 min of the session. Thus, plasma THC measurements reported herein are likely not representative of peak concentrations achieved during the session, making it difficult to directly compare our values to those obtained in human studies or rodents using different methods of drug delivery.

Although we observed diminished responding for vapor toward the end of gestation, we nonetheless observed a significant reduction in neonatal birthweight in cannabis-exposed offspring relative to vehicle-exposed offspring that was developmentally transient and not evident in adulthood. These data suggest that this pattern of use was sufficient to produce a cannabis-specific neurodevelopmental effect that is commonly observed in humans [16]. Importantly, the volitional access paradigm likely minimizes unintended stress effects due to non-contingent vapor delivery, as previous studies from our laboratory have indicated low birthweight effects in offspring from both cannabis and vehicle vapor exposure groups [45]. It should be noted that blood samples were only taken from cannabis self-administering dams – however, we attempted to minimize the potential effects of blood collection stress on developmental outcomes by taking blood samples for THC measurements prior to conception. Since this low birthweight effect has not been typically observed in studies employing forced injections of cannabinoids [2,5,20,23,38], our data argue that volitional access and the route of cannabinoid administration may be important factors to consider when interrogating long-term effects of prenatal cannabinoid exposure using animal models.

We also examined cannabis vapor self-administration in adult male and female offspring from dams that self-administered cannabis during pregnancy. Surprisingly, cannabis-exposed male, but not female, offspring made fewer active nosepokes and received fewer vapor deliveries compared to control rats, regardless of whether they were assigned to self-administer cannabis or vehicle vapor. Our data collectively indicate that prenatal cannabis exposure does not increase susceptibility for aberrant cannabis use in adulthood. These findings are in direct contrast with the extant human literature [9,12,27,34], as well as preclinical studies revealing augmented alcohol and opiate reinforcement in cannabinoid-exposed offspring [7,35,40]. They are also seemingly discordant with a growing body of evidence depicting profound alterations to the dopamine system in cannabinoid-exposed male offspring that result in a “hyperdopaminergic phenotype” [13,22,37,44] that would be expected to increase addiction susceptibility [26]. These changes include alterations in the density and function of dopaminergic neurons, as well as differences in the expression of proteins associated with dopamine receptors [13,22,37,44]. Notably, this hyperdopaminergic state was reported in juvenile rats [13]. Thus, it is possible that the behaviors observed herein may be due to protracted adaptations caused by an earlier, transient hyperdopaminergic state, and that the window for increased susceptibility for aberrant cannabis use is constrained to adolescence. We are poised to examine this possibility in future studies. It is also possible that in humans, other factors such as shared genetics and environments [6,19,24,32,48], contribute to increased risk of cannabis use rather than prenatal exposure *per se*.

It should also be noted that male cannabis-exposed offspring display attenuated responding for all vapor, regardless of whether it contained cannabis extract. Thus, it is unlikely that prenatal cannabis exposure is producing a particular aversion to cannabis, but rather is affecting vapor self-administration via some unknown third variable that is directly impacted by prenatal exposure. For instance, prenatal cannabis exposure could produce broad effects on ambulation or potentially interfere with the development of systems that are involved in novelty seeking, each of which would manifest as a decrease in nosepoke responding. Future studies will undoubtedly be needed to better understand the precise causes underlying this unexpected phenomenon.

Another unexpected finding from this study is that cannabis-exposed males showed worse discrimination for the active nosepoke during the PR challenge, regardless of whether they were responding for cannabis or vehicle vapor. Interestingly, this same effect was not seen during the multi-day self-administration paradigm. This poor discrimination index could reflect a difficulty in adapting to a more rapidly changing schedule of reinforcement. Alternatively, the increase in inactive responding among cannabis-exposed rats during the PR challenge could reflect adoption of new behavioral strategies in the face of shifting environmental contingencies. In this regard, exploring alternative strategies in response to changing reinforcement schedules could be viewed as an adaptive response. However, we have previously reported impaired behavioral flexibility in adult rats that were passively exposed to cannabis vapor in *utero* [45], which might imply greater rigidity in decision-making strategies. Future studies will be required to fully understand potential differences in cannabis-seeking strategies, as well as the extent to which these strategies contribute to the maintenance of habitual cannabis use.

Interestingly, unlike male cannabis-exposed offspring, female cannabis-exposed offspring did not differ from their control counterparts with respect to active nosepokes or vapor deliveries. There were also no differences observed between prenatal treatment groups during the PR challenge. Instead, a difference emerged in the discrimination index during the 21-day self-administration paradigm. We found that cannabis-exposed female offspring showed worse discrimination for the vapor-associated nosepoke, regardless of their assigned vapor. This may suggest that cannabis-exposed female offspring are impaired in their ability to learn the association between the active nosepoke and vapor relative to vehicle-exposed females. However, this is unlikely given that cannabis-exposed females showed robust self-administration across all reinforcement schedules and mean discrimination indices during the PR challenge exceeded the 2:1 active:inactive response ratio for every cannabis treatment group. The most reliable effect observed in female offspring was with respect to cannabis vapor self-administration, such that female rats in all prenatal exposure conditions showed higher rates of responding for cannabis vapor relative to vehicle vapor, particularly under more demanding schedules of reinforcement. This corroborates vapor self-administration from our own laboratory [15] and is supported by a growing body of literature indicating robust sex differences in the pharmacokinetic [31] and motivational [10] properties of cannabinoids. Moreover, these data are in line with a recent study revealing sex differences in conditioned place preference (CPP) for vaporized THC [51]. Specifically, whereas male rats for a CPP for both medium (5 puffs @ 200 mg/ml THC) and high (10 puffs of 200 mg/ml THC) dose regimens, female rats only form a CPP for the highest dose regimen, and CPP in general was more resistant to extinction in females relative to males [51]. The precise mechanisms underlying sex differences in the reinforcing properties of vaporized cannabis will certainly require additional study. Given the established sex differences in the pharmacokinetic properties of THC [31], another intriguing explanation could be a sex-dependent effect of prenatal cannabis exposure on the absorption, distribution, metabolism, and excretion of THC. Contributions of ovarian hormones may also be a culprit, as ovariectomy suppresses the rapid acquisition and maintenance of elevated synthetic cannabinoid administration that has been observed in preclinical self-administration studies [11].

5. Conclusion

Overall, this study serves as the first demonstration of a self-administration model for cannabinoids during the gestational period. Given the relatively stable rates of self-administration of cannabis vapor, our model could serve as a more translational approach for studying effects of maternal cannabis use in rats, rather than effects of prenatal cannabinoid exposure *per se*. With continued refinement and optimization, this model will serve as a valuable tool to investigate impacts of maternal cannabis use in both offspring and dams. Our data also reveal complex multigenerational effects of maternal cannabis use on vapor self-administration in offspring that are seemingly contrary to the current body of clinical and preclinical literature. Ultimately, we hope that application of this model will provide unique insight into the risks of using cannabis during pregnancy, which can be leveraged to inform parents and healthcare providers of its potential long-term consequences.

Declaration of Competing Interest

M.N.H. is a member of the scientific advisory board for Shoppers Drug Mart, Jazz Pharmaceuticals and Lundbeck; all other authors have no conflicts of interest.

Data Availability

Data will be made available on request.

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