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Effects of Prenatal Nicotine, THC, or Co-Exposure on Cognitive Behaviors in Adolescent Male and Female Rats

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Abstract

Introduction: Although there has been a decrease in the prevalence of tobacco smoking, exposure to nicotine during pregnancy remains a substantial problem worldwide. Further, given the recent escalation in e-cigarette use and legalization of cannabis, it has become essential to understand the effects of nicotine and cannabinoid co-exposure during early developmental stages.

Aims and Methods: We systematically examined the effects of nicotine and/or THC prenatal exposure on cognitive behaviors in male and female offspring. Dams were exposed to nicotine vape or vehicle, and oral edible THC or vehicle, throughout pregnancy. Adolescent offspring were then tested in the prepulse inhibition test, novel object recognition task, and novelty suppressed feeding task.

Results: At birth, pups from mothers exposed to nicotine vape or oral THC exhibited reduced body weight, compared to control pups. Prenatal nicotine vape exposure resulted in a decreased baseline startle reactivity in adolescent male and female rats, and in females, enhanced sensorimotor gating in the prepulse inhibition test. Prenatal nicotine and THC co-exposure resulted in significant deficits in the prepulse inhibition test in males. Deficits in short-term memory were also found in males prenatally exposed to THC, either alone or with nicotine co-exposure, and in females exposed to THC alone. Finally, in males, a modest increase in anxiety-associated behaviors was found with THC or nicotine exposure in the latency to approach a novel palatable food.

Conclusions: These studies demonstrate differential effects of prenatal exposure to e-cigarette nicotine vape and/or edible THC on cognitive function, with differing effects within male and female groups.

Implications: These studies demonstrate an impact of nicotine, THC, or co-exposure during early developmental stages in utero on behavioral outcomes in adolescence. These findings have important translational implications given the continued use of nicotine and THC containing products by pregnant women worldwide, which can be applied to support healthcare and policy efforts restricting nicotine and THC use during pregnancy.

Introduction

Billions of people are estimated to consume tobacco and nicotine worldwide, resulting in more than 5 million deaths per year with a significant negative impact on the health and economic systems of society.[1] Nicotine contained in tobacco is one of the most commonly abused substances,[2] and the use of others drugs, such as cannabis, is higher in those who already smoke nicotine cigarettes.[3,4] Of high concern, the use of these substances during pregnancy is a risk to the health of the developing fetus, since the main psychoactive agents in tobacco and cannabis, nicotine and Δ^9-tetrahydrocannabinol (THC), respectively, cross the placenta and the fetal blood-brain barriers.[5] In the brain, nicotine acts on the nicotinic acetylcholine receptors, whereas THC acts on the cannabinoid receptors. While exogenous agents may not induce obvious effects on the mother during pregnancy, they can have harmful neurological effects on the child which may not be overtly detectable at birth. Indeed, prenatal nicotine exposure has been shown to modify gene expression and has been associated with fetal brain growth restriction in some cases.[6] The functional role of cannabinoid receptors during the initial stages of embryogenesis is not well understood, but these receptors appear to be important in controlling cell proliferation and differentiation.[7] Further, both of these receptor classes exhibit overlapping expression patterns within brain regions implicated in sensorimotor processing, learning, and memory function,[8] indicating that exposure to one or both of these substances during early development may lead to differential impacts on cognitive processing.

Prenatal tobacco and nicotine exposure has been correlated with deficits on later cognitive ability, executive function, and attention deficit hyperactivity disorder (ADHD), including in children and adolescents.[9–12] The symptoms associated with ADHD often begin in early childhood, occur in all settings, and can persist into adulthood.[13] Studies in rodents have provided further evidence directly demonstrating nicotine-mediated effects on cognitive function, ADHD-like responses in attentional and emotional control, and cortical neuronal deficits.[14–16] The potential for nicotine-mediated deficits has become more concerning with the emergence of e-cigarettes, which are perceived as less harmful than tobacco cigarettes, to the point that they are thought to cause little to no adverse effects during pregnancy.[17] Indeed, e-cigarettes are currently being promoted to be used during pregnancy as a cessation aid for tobacco smoking.[17–19] Similar, albeit less consistent, reports have also emerged regarding prenatal

cannabis exposure.[20–22] For instance, infants born to mothers who used cannabis during pregnancy show increased tremors and startles, poor habituation to visual stimuli, deficits in cognitive performance, attention and short-term memory, and increased levels of impulsivity and hyperactivity.[23–28] During adolescence, fetal cannabis exposure is also associated with lower academic achievement, problems with impulse control, and poor learning and memory ability.[29–31] Taken together, these prior studies highlight the importance of examining the effects of nicotine and/or THC during different prenatal stages on later cognitive processes in adolescence.

In humans, epidemiological studies have also documented frequent co-use of nicotine and THC, and nicotine has been reported to increase the perceived effects of THC which further promotes co-use.[32–34] It has also been proposed that cannabis legalization has increased tobacco use through normalization; thus, mixing tobacco and cannabis may introduce non-tobacco users to tobacco.[35] Further, young adults who co-used nicotine and THC report heavier use and greater problematic behaviors than those who are not co-using.[36] Interestingly, exogenous cannabinoids modulate endogenous cholinergic neurotransmission,[37] and nicotine administration alters endogenous cannabinoid signaling.[38] Thus, the interaction between nicotine and THC is thought to be more complicated than previously recognized. For instance, cannabinoid receptor antagonists can decrease the release of nicotine-induced mesolimbic dopamine,[39–41] and at low doses, cannabinoid receptor agonists stimulate cortical and hippocampal acetylcholine release,[42] which may be modulated by endogenous cannabinoids.[21] Given this, controlled basic research studies are needed to systematically define the unique effects of the co-use condition on developmental processes and behavioral outcomes.

Therefore, the aim of the current study was to examine the effects of in utero nicotine vape and/or edible THC exposure on cognitive function in adolescence. These methods were utilized given the high incidence of e-cigarette nicotine and edible THC use among the general population, including pregnant women.[18,43–45] Further, since the combined impact of nicotine and THC co-exposure can exert differential developmental outcomes in utero[33], we included the co-exposure condition in consideration of the unique impact on neurodevelopment processes. In these studies, adolescent male and female offspring were examined for differences in startle reactivity, sensorimotor gating with prepulse inhibition (PPI), learning and memory with novel object recognition (NOR), and exploratory behavior with novelty suppressed feeding (NF). Findings derived from these investigations reveal important insights into the long-term impact of nicotine and/or THC resulting from drug exposure during prenatal neural development, which may thereby inform evidence-based discussions for public policy efforts.

Materials and Methods

Animals

Adult Wistar male and female rats were purchased from Charles River and bred in our colony. Prior to pairing with a male, female rats were randomly assigned to group conditions and then exposed to the drug for 5 consecutive days, consistent with group assignment. The four exposure group conditions consisted of: Control (vehicle vapor and vehicle oral); THC (vapor vehicle and THC oral); NIC (vapor nicotine, vehicle oral); and NIC/THC (vapor nicotine, THC oral). Thereafter, on exposure day 5, the dams were paired with a stud male ~5 h after the daily drug exposure. This allowed for a pre-exposure period in the females to more closely mimic drug use in humans with initiation prior to pregnancy. After confirmation of successful mating with the presence of the vaginal plug, the stud male was removed, and female subjects continued the drug treatment until gestational day (GD) 20. On each exposure day, THC (5 mg/kg) or sesame oil vehicle was first administered via oral gavage immediately prior to the daily vapor session.[46–48] The volume of THC was adjusted each day to account for increased daily weights resulting from pregnancy. The oral THC dose was selected as it is considered to be equivalent to moderate human gestational exposure.[49,50] E-cigarette vapor, either with or without nicotine (5 mg/ml), was administered into the chamber across 1 h with 5-sec vapor puffs at 5 min intervals, resulting in 12 total puffs per 1-h session in sealed chambers (340 mm × 237 mm × 198 mm) (La Jolla Alcohol Research, LJARI). The Med Associates custom computer interface allows for the delivery of vapor under specified controlled conditions (temperature 400°F; 5-s programmed puff, total vapor time in chamber per puff ~100 s). The positive-pressure chamber air flow was vacuum controlled to maintain air delivery through the intake valve at 1 L/min. This nicotine vape dose exposure paradigm was employed to provide a concentration >22 ng/ml blood cotinine levels, as described previously.[51,52] Dams and pups were not exposed to any drug following parturition since we sought to limit stress-related effects on early developmental processes, which could have confounded our findings. The stud males used for the mating (n = 7) were used in rotation in a random assignment. At weaning (PND 21), offspring subjects (male and female) were housed 2–3 per cage, and n = 4 dams per group treatment were used for each cohort. A total of 4 cohorts of experimental subjects were used across all the tasks. Subjects were tested as follows: Cohort 1: PPI; Cohort 2: NOR and NF; and Cohorts 3 and 4: PPI, NOR, and NF. Based on the PND requirement of testing, we were unable to accommodate cohorts 1 and 2 with the alternate behavioral measures because of daily testing time limitations in the experimental room, with subjects being tested only during the dark phase of the light cycle. All breeders and experimental animals were maintained in the environmentally controlled vivarium on a 12h:12h reversed light:dark cycle. Food and water were provided ad libitum, except as noted below for the NF test. The vivarium was maintained at a constant temperature of 22°C and relative humidity of 65%. All procedures were conducted in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of The University of California, Irvine.

Drugs

Δ^9-tetrahydrocannabinol (THC) was obtained in ethanol (NIDA Drug Distribution Program). To prepare the drug for administration, the ethanol was evaporated under nitrogen, and the remaining THC was dissolved in vehicle (sesame oil) for a concentration of 15 mg/ml, which was then sonicated for 15 min; for each dam, the volume was adjusted to provide 5 mg/kg THC orally based on each subjects' daily weight. (-)-Nicotine hydrogen tartrate salt (MP Biomedicals) was dissolved in vehicle (50:50 propylene glycol and vegetable

glycerin) for a dose of 5 mg/ml (freebase, pH 7.4) and administered via aerosolization with the e-cigarette equipment (LJARI).

Prepulse Inhibition

Startle and prepulse inhibition (PPI) testing were conducted in startle chambers (SR-LAB, San Diego Instruments), using an experimental design as previously described.[53,54] The rodent's startle response data is directly recorded by an accelerometer that produces an analog output voltage signal in millivolts (mV). The rodent startle response is all 4 limbs crouching dynamically, with force of the movements generating voltage recorded by the accelerometer. Each chamber consisted of a Plexiglas cylinder, 5 cm in diameter, resting on a platform in a ventilated sound-attenuating chamber. Speakers mounted 33 cm above the cylinders produced the acoustic stimuli, and movements of the animal were transduced by piezoelectric accelerometers mounted under the cylinders and stored by the computer interface. At PND 22, rats ($n = 48$ female, $n = 41$ male) were placed into the startle chambers, and testing was initiated after a 5 min acclimation period at a 60 dB background noise level. The PPI test protocol then consisted of a startle block comprising five 120 dB startle pulses, followed by five prepulse blocks, which consisted of a 120 dB startle pulse preceded by either at 70 dB prepulse, 75 dB prepulse, 80 dB prepulse, or 85 dB prepulse. Each block presented the specific prepulses in a randomized manner. Each prepulse was 20 ms in duration, followed by 50 ms interstimulus interval, and then 40 ms of the 120 dB startle pulse. Prior to each block after the first, subjects were exposed to a baseline level of 60 dB for 100 ms. To calculate percent PPI, the following equation was used: percent PPI = [(S—P)/S] × 100, where S is the average baseline startle response per subject (mV) and P is the average response following each prepulse and startle pairing (mV). Thus, a lower value of percent PPI indicates a greater behavioral movement in the presence of a prepulse, thereby demonstrating a deficit in sensorimotor gating; conversely, a higher percent PPI is indicative of more efficient sensorimotor gating with an attenuated movement for the prepulse-startle pairing.

Novel Object Recognition

At PND 35, male ($n = 42$) and female ($n = 40$) subjects were examined in the novel object recognition (NOR) task, which is a robust and reliable procedure commonly used in the field to assess recognitive memory in rodents.[55–57] The task is based on the observation that rodents exhibit a greater tendency to interact with a novel object, as compared to a familiar object.[56] Thus, this test examines the animal's preference behavior to interact with the two objects. The two objects are placed equidistance apart from the edges and each other in an open field arena composed of plexiglass (35 cm L × 35 cm W × 31 cm H). A shielded white light lamp is located ~90 cm above the apparatus to provide consistent lighting, and a video camera is mounted above the arena for behavioral recording. The procedure consists of three phases: (1) habituation (5 min), (2) pretest (10 min), and (3) test (10 min). During the habituation phase, the animals are permitted to acclimate to the open field arena by freely moving around the open space. In the pretest, two identical objects (A + A) are positioned at equal distance from the wall of the arena and between each other, leaving the animal freely to move around, explore and interact with both objects. Subjects are

then placed back into their home cage for 2 h to assess short-term memory. During the test phase, one of the two objects (A) is substituted with a novel object (B), that differs in color and shape (unfamiliar). The location of the familiar versus unfamiliar object was counterbalanced in the two placement areas as described above. Our pilot studies demonstrated no preference for either object under baseline, novel conditions. Consistent with NOR test criteria,[58] subjects were excluded if they did not explore the objects for >30 sec of the test time ($n = 1$ control, 3 THC, and 3 NIC/THC females). Locomotor activity recorded by the video camera was scored with ANY-Maze Software (Stoelting Co.). Experimenters blinded to the group conditions scored the time each subject spent directly sniffing the objects, and exploration time was calculated for both objects. Discrimination index (DI) preference was calculated according to the following equation: DI = (time exploring object B—time exploring object A) ÷ (total time exploring both objects), in which a value >0 indicates more time spent on the novel object B, and <0 indicates more time spent on the object A.[57,59] Thus, in these studies, short-term recognition memory is evidenced by positive DI values.

Novelty Suppressed Feeding Test

The avoidance of unfamiliar food, neophobia, is essential for survival because consumption of a new food could lead to illness or even death.[60] Therefore, the novelty suppressed feeding test (NF) is considered a measure of anxiety-related behavior.[60,61] At PND 37, subjects ($n = 47$ female, $n = 42$ male) were examined in this task, which includes 2 phases: (1) habituation (5 min) and (2) food interaction and consumption phase (10 min). The animals were food restricted ~8 h prior to the beginning of the test, which has been found to be sufficient to induce food-seeking behaviors for palatable food while not inducing significant weight loss. The open field arena is composed of plexiglass (35 cm L × 35 cm W × 31 cm H), with a shielded white light lamp ~90 cm above the apparatus for consistent lighting, as described previously.[62] Subjects are placed into the center of the apparatus and video recorded by a camera suspended above the open field. For the habituation phase, subjects are permitted to acclimate to the arena by freely roaming for 5 min. Thereafter, the food interaction and consumption phase begins with the novel food pellets being placed in the center of the arena in glass petri dish (Chocolate food pellets 5TUL, 45 mg, Test Diet). The food was weighed prior to and after the test phase to quantify mg consumption. Experimenters blinded to the group conditions scored the videos by quantifying latency to approach the food pellets and latency to begin food consumption.

Statistical Analyses

Data were analyzed by a one-way or repeated measures two-way analysis of variance (ANOVA), followed by Tukey or Dunnet post hoc test with correction for multiple comparisons (GraphPad Prism), as appropriate. Drug exposure was administered to the dams during pregnancy to induce distinctly different effects on neurodevelopment based on the treatment condition (Vehicle, NIC, THC, and NIC/THC). Since each group represents a unique, independent developmental state at the testing timepoint in adolescence,[63,64] one-way ANOVA comparisons were performed with each treatment represented as an independent group. Further, these studies were conducted with the a priori experimental design to examine for differences independently in males and females, and as

such, no direct comparisons were conducted between sexes. The criterion for significance was set at $p < .05$.

Results

### Effects of Prenatal Drug Exposure on Litter Number and Postnatal Weight

Female dams were randomly assigned to groups and exposed to drug treatments 5 days prior to mating and throughout gestational days (GD) 1-20 (Supplementary Fig. 1a). To first evaluate if the pregnancy treatment affected litter size, the total number of offspring for each dam was quantified at postnatal day (PND) 1, but no significant differences in litter size were found among the groups (One-way ANOVA, $F_{(3,10)} = 0.3650$, $p = .7798$, $R^2 = 0.0987$) (Supplementary Fig. 1b). To determine if prenatal drug treatment altered general growth, pups were weighed at the following PND timepoints: PND1, PND15, and PND21. Interestingly, a significant group difference was found at PND1 (One-way ANOVA, $F_{(3,77)} = 14.89$, $p < .0001$, $R^2 = 0.3671$); the post hoc analysis revealed that the pups prenatally exposed to THC ($p < .0001$) or NIC ($p = .0340$) weighed significantly less than the control group (Supplementary Fig. 1c). However, differences among the groups were no longer present at PND15 (One-way ANOVA, $F_{(3,77)} = 1.924$, $p = .1327$, $R^2 = 0.0697$) (Supplementary Fig. 1d)

or at PND21 (One-way ANOVA, $F_{(3,77)} = 1.582$, $p = .2005$, $R^2 = 0.0581$) (Supplementary Fig. 1e).

Effects of Prenatal Drug Exposure on Sensorimotor Gating in Adolescence

In the PPI procedure, a lower percent PPI is indicative of decreased sensorimotor gating following the prepulse that was paired with a startle pulse. To examine the effects of nicotine and/or THC during pregnancy on later adolescent behavior, we conducted the PPI test and assessed both initial startle reactivity and percent PPI.[65] At PND22, analysis of the baseline startle response was performed, followed by the prepulse and startle pulse paired sequences, with the prepulse of varying decibels (dB). Adolescent male rats were found to exhibit differences in the initial baseline startle response across groups (One-way ANOVA, $F_{(3,37)} = 3.786$, $p = .0183$, $R_2 = 0.2349$) (Figure 1, a). Specifically, the post hoc test revealed that prenatal NIC treatment led to reduced startle reactivity versus the control ($p = .0127$). It was also noted that prenatal NIC/THC co-exposure led to a potentially lower startle reactivity in males, although this did not reach statistical significance ($p = .0713$). Next, we examined the overall percent PPI across all of the prepulse administrations in males and found a significant decrease in the NIC/THC co-exposure group relative to the NIC group (One-way ANOVA, $F_{(3,37)} = 3.478$, $p = .0254$, $R^2 = 0.2200$; post hoc, NIC/THC
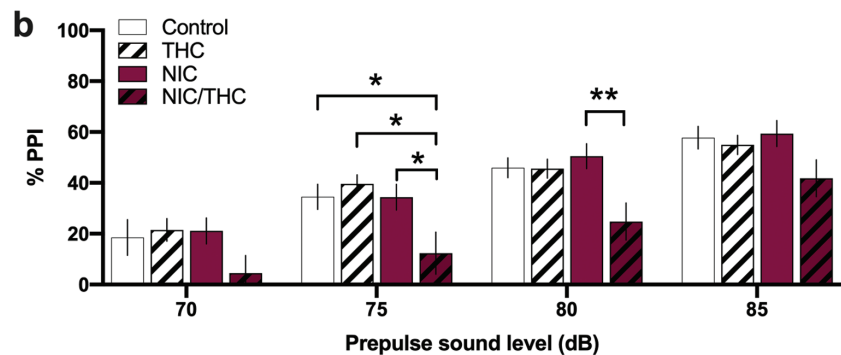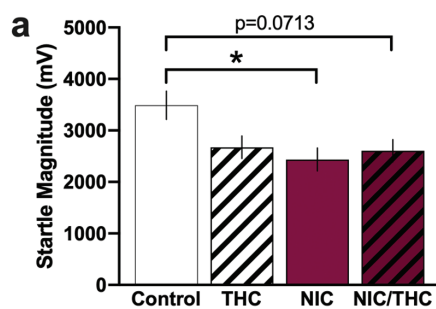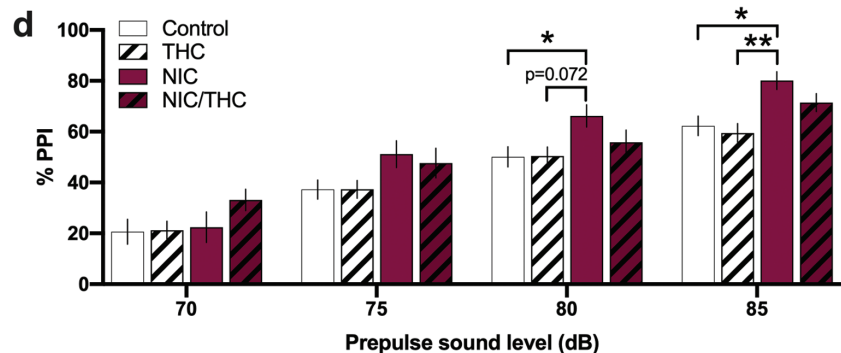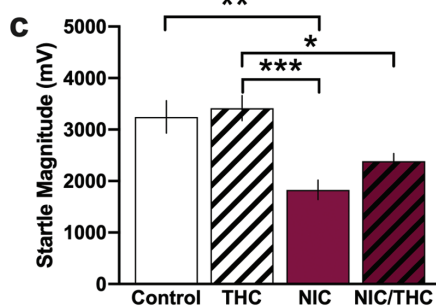

Figure 1. Altered sensorimotor gating in male and female adolescent offspring following prenatal nicotine and/or THC exposure. (a–b) Following prenatal exposure to THC, NIC, NIC/THC, or vehicle control, male offspring ($n = 8–14$/group) were examined for startle reactivity and prepulse inhibition (PPI) at PND22. (a) Prenatal NIC treatment resulted in a decrease in startle reactivity compared to the control. Similar effects were found in the NIC/THC co-exposure condition, but this did not reach statistical significance. (b) Following the 75 decibel (dB) prepulse, males exposed to NIC and THC exhibited a deficit in percent PPI compared to all other groups. The decreased response for the NIC/THC co-exposure group persisted at the 80 dB prepulse, when compared to the nicotine group. (c–d) Female offspring ($n = 10–15$/group) were examined for startle reactivity and PPI at PND22. (c) Adolescent females prenatally exposed to NIC exhibited a significant decrease in startle reactivity compared to the THC and control groups. (d) When specifically examined across the differing prepulse auditory levels, prenatal NIC treatment resulted in enhanced sensorimotor gating compared to the control group at 80 dB, and control and THC groups at 85 dBs, in females. *$p < .05$, **$p < .01$, and ***$p < .001$. Data represent mean ± SEM.

vs. NIC p = .0270) (Supplementary Fig. 2a). Baseline movements during habituation at 60 dB were also examined, but male subjects did not differ among groups (Mean mV ± SEM: Control 17.04 ± 2.582; THC 16.13 ± 1.644; Nicotine 12.25 ± 0.986; NIC/THC 16.45 ± 1.498; One way-ANOVA, $F_{(3,37)}$ = 2.126, p = .1135, R^2 = 0.147). However, when examining across the specific paired prepulse dB levels, group differences were revealed (Repeated measures two-way ANOVA, *Treatment* $F_{(3,37)}$ = 3.479, p = .0254, *Intensity* $F_{(3,111)}$ = 136.2, $p < .0001$, *Interaction* $F_{(9,111)}$ = 1.198, p = .3031) (Figure 1, b). The post hoc test indicated that the prenatal NIC/THC led to an attenuation of percent PPI in adolescent males. Specifically, at 75 dB, the NIC/THC co-exposure condition was significantly less than the control (p = .0458), THC (p = .0109), and nicotine (p = .0220) groups. At the 80 dB prepulse, the NIC/THC group was significantly less than the NIC group (p = .0050).

For the adolescent females, differences were also found in the baseline startle response across groups (One-way ANOVA, $F_{(3,44)}$ = 8.366, p = .0002, R^2 = 0.3632) (Figure 1, c). The post hoc test revealed that the prenatal NIC group exhibited an attenuated startle response compared to the control (p = .0011) and THC (p = .0006) groups, and the NIC/THC co-exposure group was also lower than THC (p = .0417). Next, the overall percent PPI accumulated across all prepulse dBs was examined, and no significant differences were found among groups (One-way ANOVA, $F_{(3,44)}$ = 2.728, p = .0553, R^2 = 0.1568) (Supplementary Fig. 2b). Baseline movements during habituation at 60 dB were also examined, and female subjects did not differ among groups (Mean ± SEM: Control 15.52 ± 1.406; THC 13.59 ± 1.279; NIC 15.59 ± 2.205; NIC/THC 17.66 ± 2.850; One-way ANOVA, $F_{(3,44)}$ = 0.6606, p = .5807, R^2 = 0.0431). However, when the percent PPI was examined with the specific prepulse dBs, group differences emerged (Repeated measures two-way ANOVA, *Treatment* $F_{(3,44)}$ = 2.912, p = .0448, *Intensity* $F_{(3,132)}$ = 227.9, $p < .0001$, *Interaction* $F_{(9,132)}$ = 2.969, p = .0030) (Figure 1, d). The post hoc test revealed significant differences with both the 80 and 85 dB prepulses. Specifically, at 80 dB, an increase in the percent PPI was found for the NIC group as compared to the control (p = .0394), with a trend for a difference with the THC group (p = .0722). At 85 dB, the prenatal NIC exposure group was significantly higher than the control (p = .0178) and THC (p = .0089) groups.

Effects of Prenatal Drug Exposure on Short-term Memory and Exploratory Behavior in Adolescence

At PND35, subjects were examined in the NOR task, in which the amount of time exploring a novel object provides an index of recognition memory[66] and can be defined as short-term memory with the 2 h delay imposed in the current studies.[67] The discrimination index was calculated to determine preference for the novel object (represented as positive values) as compared to preference for the familiar object (represented as negative values). The male THC and NIC/THC groups appeared to present a negative discrimination index, suggesting no preference for the novel object. When statistically analyzed, significant differences were found among the male groups in the discrimination index (One-way ANOVA, $F_{(3,38)}$ = 6.020, p = .0018, R^2 = 0.3222) (Figure 2, a). The post hoc test revealed statistically significant differences between the control group versus the prenatal THC (p = .0017) and NIC/THC (p = .0073) exposure groups. In adolescent female rats, a similar effect with prenatal THC exposure was observed, in which the control significantly differed from the prenatal THC exposure group (One-way ANOVA, $F_{(3,36)}$ = 3.153, p = .0365, R^2 = 0.2081; post hoc test, control vs. THC p = .0113) (Figure 2, b). Next, the percentage of time spent exploring both objects was examined to determine if behavioral or exploratory deficits were present among groups. No significant differences were found in time devoted to exploring both of the two objects during the pretest and test phases for males (Repeated measures two-way ANOVA, *Treatment* $F_{(3,38)}$ = 1.550, p = .2175, *Session* $F_{(1,38)}$ = 1.505, p = .2275, *Interaction* $F_{(3,38)}$ = 2.186, p = .1056) (Supplementary Fig. 3a) and females (Repeated measures two-way ANOVA, *Treatment* $F_{(3,36)}$ = 0.8344, p = .4838, *Session* $F_{(1,36)}$ = 1.225, p = .2757, *Interaction* $F_{(3,36)}$ = 2.201, p = .1048) (Supplementary Fig. 3b). Finally, distance travelled during the full 10 min of each testing session was calculated to further discern if the groups differed in their general locomotor behavior during the sessions. This control measure was focused on assessing if motor deficits were present in any of the groups, which could have prevented them from moving around the chamber to explore
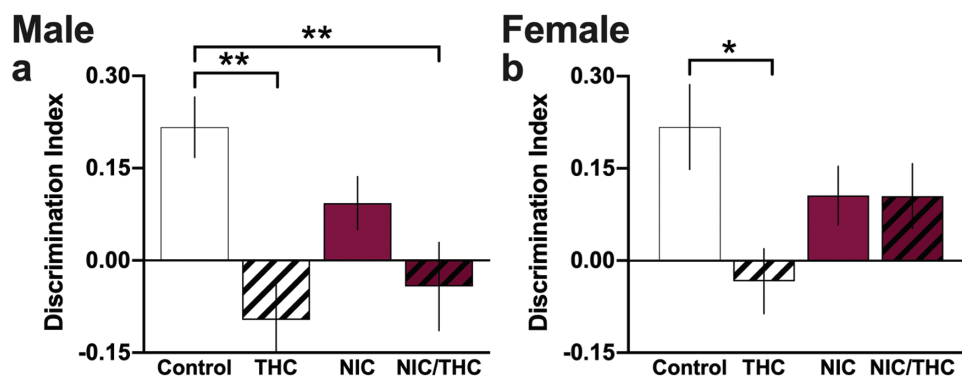

Figure 2. Prenatal THC exposure results in deficits in short-term memory during adolescence in males and females. At PND35, males (*n* = 7–16/group) and females (*n* = 8–12/group) were tested in the novel object recognition (NOR) task following prenatal exposure to THC, NIC, NIC/THC, or vehicle control. (a) Males prenatally exposed to THC or NIC/THC co-exposed show a discrimination index < 0, indicating a deficit in recognition of a novel object. Both of these groups significantly differed from the control group. (b) Females prenatally exposed to THC exhibited no discrimination between the novel and familiar object, which was evidenced as a deficit in the behavioral expression of memory as compared to the control group. *p < .05, **p < .01. Data represent mean ± SEM.

the objects. While we found no deficits among the groups, we did observe an increase in distance traveled with the prenatal THC treatment group relative to the control males, but only for the test phase (Repeated measures two-way ANOVA, *Treatment* $F_{(3,38)}$ = 3.039, *p* = .0406, *Session* $F_{(1,38)}$ = 51.03, *p* < .0001, *Interaction* $F_{(3,38)}$ = 3.850, *p* = .0168; post hoc, *Test session* control vs. THC *p* = .0088) (Supplementary Fig. 3c). The female groups did not differ in distance traveled during both the pretest and test sessions (Repeated measures two-way ANOVA, *Treatment* $F_{(3,36)}$ = 0.2403, *p* = .8676, *Session* $F_{(1,36)}$ = 11.18, *p* = .0019, *Interaction* $F_{(3,36)}$ = 0.0918, *p* = .9641) (Supplementary Fig. 3d).

Effect of Prenatal Drug Exposure on Anxiety-associated Behavior in Adolescence

At PND37, rats were examined for their avoidance of unfamiliar palatable food in an open field, which is considered an anxiety-related measure.[60,68] We first quantified latency to approach the food pellet in the center of the open field. In males, differences were observed among the groups (One-way ANOVA, $F_{(3,38)}$ = 4.480, *p* = .0087, R^2 = 0.2613) (Figure 3, a). In the post hoc analysis, we found a significantly delayed latency to approach the food pellet in the groups treated prenatally with THC (*p* = .0060) or NIC (*p* = .0439), as compared to the control group. However, in females, no differences were found among the groups in the approach latency (One-way ANOVA, $F_{(3,43)}$ = 2.058, *p* = .1199, R^2 = 0.1255) (Figure 3, b). In addition to assessing the initial approach latency, the time to consume the food pellet and amount of food consumed were assessed; these further control measures provide an indication of the hunger-induced motivation to consume food to ensure groups did not differ in their innate physiological drive to eat. Importantly, no significant differences were found across groups in the latency to consume food for males (One-way ANOVA, $F_{(3,38)}$ = 0.3205, *p* = .8105, R^2 = 0.02468) (Supplementary Fig. 4a) and females (One-way ANOVA, $F_{(3,43)}$ = 0.5310, *p* = .6634, R^2 = 0.03573) (Supplementary Fig. 4b). Further, there were no statistically significant differences in the total amount of food consumed during the session for males (One-way ANOVA, $F_{(3,38)}$ = 1.998, *p* = .1307, R^2 = 0.1362) (Supplementary Fig. 4c) and females (One-way ANOVA, $F_{(3,43)}$ = 2.485, *p* = .0734, R^2 = 0.1478) (Supplementary Fig. 4d).

Discussion

In these studies, we examined the effects of e-cigarette nicotine vape and edible THC during pregnancy on cognitive outcomes in male and female offspring. We found significant differences in the impact of single or co-exposure across multiple behavioral measures in adolescence, supporting the relevance of the prenatal environment on long-term neurodevelopmental effects, consistent with evidence in humans.[9–12,23–31] Specifically, our studies demonstrate that prenatal nicotine vape exposure led to a decrease in baseline startle reactivity in adolescent male and female rats, and in females, enhanced sensorimotor gating in the PPI test. Prenatal NIC/THC co-exposure resulted in significant deficits in the PPI test in males. Deficits in short-term memory were also found in males prenatally exposed to THC, either alone or with NIC co-exposure, and in females exposed to THC alone. Finally, a modest increase in anxiety-associated behaviors was found in males with THC or NIC exposure with the latency to approach novel palatable food, although additional measures will be necessary to fully support the anxiety-related outcome. Taken together, these findings provide evidence to support the contention that prenatal drug exposure exerts significant effects on the developing brain in utero, leading to differential long-term cognitive effects within male and female adolescent offspring.

Prenatal Impact on Sensorimotor Reactivity and Gating

During adolescence, males and females exposed prenatally to nicotine vape exhibited a decreased baseline startle reactivity to 120 dB, suggesting altered sensory and/or fear processing. Lesser effects were found in adolescent rats co-exposed to NIC/THC, and no differences were found with THC alone, suggesting the observed differences were mainly driven by the presence of nicotine during the prenatal period. Interestingly, previous studies have associated a high baseline startle magnitude with anxiety and fear-related disorders in humans.[69,70] To our knowledge, the current study is the first to report baseline differences in startle reactivity after prenatal treatment with nicotine and/or THC. Importantly, these startle effects were not because of altered general behavioral capability, as no differences were found in generalized locomotion in subsequent
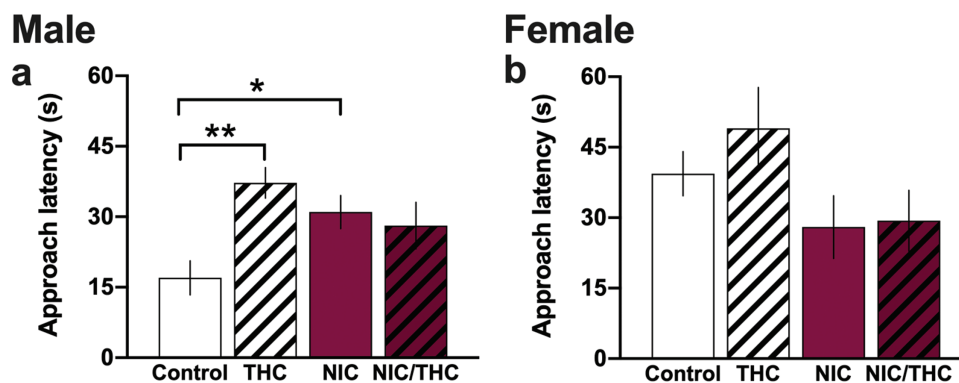

Figure 3. Longer approach latency in the novelty suppressed feeding task (NF) following prenatal THC or nicotine exposure in adolescent male, but not female, rats. At PND37, subjects were tested in the NF task following prenatal exposure to THC, NIC, NIC/THC, or vehicle control. Behaviors were examined after palatable food was placed in the center of an open field for food restricted male (*n* = 8–15/group) and female (*n* = 10–14/group) adolescent rats. (a) Males exposed prenatally to THC or NIC, but not NIC/THC co-exposure, exhibited a greater latency to approach the novel food than subjects in the control condition. (b) Female groups did not significantly differ in the latency to approach the novel food. s = seconds. **p* < .05 and ***p* < .01. Data represent mean ± SEM.

tests. Thus, future studies are needed to localize the contributing central and/or peripheral signaling mechanisms mediating the differences in startle reactivity following in utero nicotine exposure.

In regard to the sensorimotor gating response, prenatal NIC/THC co-exposure resulted in a deficit in PPI at the moderate prepulse levels of 75 and 80 decibels in male subjects, effects which were not found with THC or NIC alone. These findings suggest that nicotine and THC act synergistically in utero to alter brain circuitry involved in mediating sensory processing and the gating response in male subjects. Indeed, the neural substrates that regulate PPI overlap substantially with the brain circuitry that mediates the reinforcing properties of drugs of abuse; for instance, forebrain circuitry involved in connecting the limbic and cortico-pallido-striato-thalamic brain regions, which involve the neurotransmitters dopamine and serotonin.[71–73] In addition, estrogen has been shown to act on the dopaminergic system to regulate PPI.[74] Since serum concentrations of 17β-estradiol and testosterone can vary starting from PND4 in male and female rats,[75] differences in PPI found during adolescence may reflect activational effects of steroid hormones, in addition to the impact of THC and/or nicotine on brain systems earlier in utero. We also found that THC and NIC/THC co-exposure did not alter PPI in females, but rather, NIC vape exposure alone led to enhanced PPI sensorimotor gating at the moderate and higher prepulse decibels of 80 and 85. Our data are in contrast with a prior study demonstrating that prenatal nicotine reduced PPI in female, but not male, Sprague-Dawley rats,[76] however, the prior study employed a high dose of constant administration of nicotine via minipump during the prenatal period. In contrast, in our study, we employed an e-cigarette vape protocol to more closely mimic human patterns of drug inhalation. Interestingly, Lacy and colleagues showed that intravenous gestational nicotine does not produce alterations to sensorimotor gating at PND 14, but a significant increase was found at PND 18 and PND 75,[77] which was conducted in mixed male–female groups of Sprague-Dawley rats; these data are consistent with our findings in females that were tested at PND22. Finally, a prior study of THC prenatal exposure found sensorimotor-gating deficits in male, but not female, adult Sprague-Dawley rats (PND 60-70; 2 mg/kg THC was administered subcutaneously),[78] whereas we did not find any differences with oral THC administration in our male subjects. Thus, the differences between studies may be attributed to dose or duration of nicotine or THC and/or genetic background (Wistar vs. Sprague-Dawley), but these possibilities will need to be more systematically defined in future studies.

Prenatal Impact on Adolescent Memory

In humans, acute administration of cannabis has been shown to impair episodic memory, whereas nicotine can improve working memory performance.[79] Here, we documented short-term memory deficits in mid-adolescence (PND 35) following prenatal THC treatment in both males and females. It has been previously shown that perinatal exposure to THC can induce short- and long-term memory impairment in adult PND 80 male rats,[80] and as such, the current findings are consistent with this prior literature. However, importantly, we further demonstrated that similar deficits can also be found in the THC-exposed female offspring. We further found that similar memory deficits were present with NIC/THC co-

exposure in males, but not females, which suggests that nicotine may counteract THC's effects in the developing female brain with the co-exposure condition. Although a prior report found that in utero nicotine exposure can lead to deficits in attention and working memory in male, but not female offspring, in early adolescence (PND 21),[14] we did not find any differences with nicotine vape alone; this discrepancy could have been due to variations in nicotine dose, exposure duration, and/or age at testing. Thus, it will be of interest in further studies to determine the effects of nicotine vape and/or edible THC at additional developmental stages.

Importance of Examining Nicotine and THC Co-Use

Interestingly, we predicted that NIC/THC co-use would result in synergistic effects in these outcome measures given the overlapping patterns of nicotinic acetylcholine and cannabinoid signaling mechanisms in the brain. This was evident in adolescent males with PPI, in which NIC/THC co-exposure elicited a behavioral deficit that was not observed in the presence of either nicotine or THC alone, thereby indicating that co-exposure can be particularly detrimental for this outcome measure. However, across other measures, the co-use condition resulted in either similar effects as either nicotine or THC alone, or no difference when compared to the control group. These two differing outcomes have interesting implications. First, when the co-use condition results in similar effects as single drug exposure, one may conclude that the primary drug induced the effects found in the co-exposure condition; for instance, in males, THC led to a deficit in novel object discrimination and this effect was similar to that found with NIC/THC co-exposure, suggesting THC was the main driver of these effects. On the other hand, when administered together, nicotine and THC appeared to counteract the effects of one of the other drugs in some cases; for instance, in females, prenatal nicotine exposure resulted in an enhanced PPI sensory gating response, which was ameliorated in the co-exposure group. These findings indicate that the presence of THC opposed the developmental effects of prenatal nicotine for this outcome measure.

Relevance to Human Studies

The translational utility of the PPI assessment is attributed to many studies that have found patients suffering from neurological and psychiatric disorders exhibit deficits in such sensorimotor gating, including schizophrenia, Huntington's disease, and Alzheimer's disease.[81–84] In addition to differences in the PPI response, baseline startle reactivity has also been shown to be altered in patients, for instance in individuals with autism or panic disorder.[85,86] Therefore, the current studies provide evidence that prenatal NIC/THC exposure in males may contribute to developmental alterations in processes underlying sensorimotor gating with PPI response, which could also be associated with psychiatric disorders. The effect of the co-exposure condition on males was also evidenced as deficits in the NOR task, supporting the contention that drug co-use can lead to adverse outcomes across multiple measures. However, as noted above, the NOR behavioral differences may have been primarily driven by the actions of prenatal THC, which resulted in similar effects as the NIC/THC co-exposure condition. Therefore, future studies are needed to disentangle the neurodevelopmental effects more clearly at the synaptic level. The current studies also provide further evidence that the impact of prenatal drug exposure

can extend to anxiety-related effects in males, in which nicotine or THC alone during the prenatal period increased the latency to approach a novel food stimulus in the adolescent males; one potential limitation with this conclusion is that motivational differences may underlie such food-driven behavior, but it should be noted that these groups did not differ in the latency to consume nor in the total amount of food consumed. However, it would be of benefit in further studies to define the strength of this effect more rigorously across multiple anxiety-related behavioral measures at the same adolescent age. Of further relevance, other studies in humans have demonstrated that allelic variation in the catechol-O-methyltransferase gene, when combined with adolescent cannabis use, may predispose individuals to develop symptoms associated with schizophrenia.[87,88] Therefore, the intersection of genetics and prenatal drug exposure will be important to investigate in future studies when defining drug-related impacts on neurodevelopment processes in humans.[89]

Experimental Limitations

When studying the effects of prenatal nicotine and/or THC exposure in rodents, such as the studies described herein, experimental limitations must be considered for potential translation to the human condition. First, in the current studies, dams were treated with nicotine and/or THC prior to and during pregnancy. The 2 weeks after birth in rodents has been proposed to be representative of the third trimester of fetal brain development in humans,[90] but the subjects in our studies were not exposed to drug during this postnatal period. The decision to not treat the mothers after parturition was implemented since continued treatments would have required maternal separation for drug exposure to be conducted in the vapor boxes. Importantly, maternal separation has been shown to induce significant stress-related effects, which could have resulted in differential outcomes in the offspring,[91,92] thereby likely confounding interpretations for the consequences of in utero drug exposure. Furthermore, prior literature suggests that cannabis use among women is more prevalent in the first and second trimesters of pregnancy.[44] Another consideration in this regard is the effects of maternal care, either during drug exposure or during withdrawal, on offspring development. Our experimental conditions represent the withdrawal condition for maternal care since drug exposure ended with parturition. We chose this approach since (1) women may stop using drugs in the third trimester or after the birth of a child,[44] (2) shorter durations of breast feeding have been found in women using cannabis,[44] and (3) THC can exert effects on prolactin levels that are involved in mediating lactation which may differentially affect nutrition being provided to the pups.[93] Thus, additional studies are necessary to represent all possible maternal care situations. For instance, it would be interesting in future studies to examine the interaction of maternal separation stress and continued drug exposure following birth on the development of the offspring. Next, the current investigations were focused on maternal drug use, but paternal drug use may be equally impactful on neurodevelopmental processes via epigenetic changes in sperm. This has been demonstrated previously with nicotine exposure exerting a multigenerational impact on offspring susceptibility with stress-related behaviors, HPA-axis dysregulation, nicotine self-administration, and cognitive flexibility,[94,95] and with paternal THC exposure inducing significant impairment in the offspring in an operant attention task.[96] Thus, it will be interesting in further studies to determine the impact of combined maternal and paternal drug exposure on offspring development.

Conclusions

Drug use during pregnancy results in several cognitive effects in sensory processing, sensory gating, and learning and memory, which may contribute to susceptibility for symptomology associated with mental illness in later life. Here, we found that these effects can be detected during adolescence, with differential findings within males and females. It will be important in future studies to disentangle the specific mechanistic contributions underlying the impact on the developing neurocircuitries of the prenatal brain, particularly since these findings may extend to other drugs that act on the nicotinic acetylcholine receptors (including cessation therapeutics, such as varenicline and nicotine replacement therapy) and cannabinoid receptors (including cannabidiol). In sum, our findings thereby support the importance of deterring pregnant women from partaking in nicotine and cannabis use to protect the neurological development of their children.

Supplementary Material

A Contributorship Form detailing each author's specific involvement with this content, as well as any supplementary data, are available online at https://academic.oup.com/ntr.

Supplementary Fig. 1. Prenatal exposure to e-cigarette nicotine vape and/or edible THC. (a) Schematic illustrating experimental design with prenatal exposure of the female dams to THC, NIC, NIC/THC or vehicle control. Female dams began drug exposure 5 days prior to mating. Drug exposure continued to be administered to the pregnant dams across GD 1 to GD 20 in accordance with group assignment. At PND 21, pups were separated from the mother and divided by sex into 2-3 per cage. At PND 22 rats were assessed in the PPI test. At PND 35, the NOR was performed, and finally, at PND 37, subjects were examined with the NF test. Schematic created with BioRender.com. (b) The number of pups in each litter were quantified on PND 1, with no differences among treatment groups. (c-e) Pups (n = 16-24/group) were weighted at PND 1 (c), PND 15 (d) and PND 21 (e). At PND 1, subjects exposed to THC or NIC, but not NIC/THC co-exposure, weighed less than the control group. Differences among groups were not found at PND 15 or PND 21. *p < .05, ****p < .0001. Data represent mean ± SEM.

Supplementary Fig. 2. Overall sensorimotor gating averaged across prepulse decibels in male and female adolescent offspring. Following prenatal exposure to THC, NIC, NIC/THC or vehicle control, male (n = 8-14/group) and female (n = 10-15/group) were examined for PPI at PND 22. Primary data are presented in Fig. 1. (a) When examining the overall percent PPI across all prepulse-startle pairings, males co-exposed to NIC/THC prenatally exhibited decreased sensorimotor gating compared to the control group. (b) Females did not differ in the overall mean percent PPI among groups. *p < .05. Data represent mean ± SEM.

Supplementary Fig. 3. Prenatal drug exposure did not alter the percent of time exploring both objects and general locomotion during the NOR task. At PND 35, males (n = 7-16/group) and females (n = 8-12/group) were tested in the NOR task following prenatal exposure to THC, NIC, NIC/THC

or vehicle control. (a) Male subjects across groups did not differ in the total percentage of time exploring both objects during the pretest or test sessions. (b) Female subjects across groups did not significantly differ in time spent exploring both objects during the pretest and test sessions. (c-d) Subjects were also examined for the distance traveled during both phases of the task, as a control for general locomotion based on prenatal group exposure. (c) In males, groups did not differ during the pretest, but increased distance traveled was found for the THC group during the test session. Interestingly, this was in contrast to the decreased discrimination index for the novel object found during the test phase in Fig. 2a, indicating that the discrimination deficit was not due to general behavioral inhibition based on prenatal drug exposure. (d) Female subjects did not differ in general locomotion during adolescence across groups. *p < .05. Data represent mean ± SEM.

Supplementary Fig. 4. No differences in the latency to eat or in the total amount of food consumed in the NF task following prenatal drug exposure. At PND 37, subjects were tested in the NF task following prenatal exposure to THC, NIC, NIC/THC or vehicle control. Behaviors were examined after palatable food was placed in the center of an open field with food restricted male (n = 8-15/group) and female (n = 10-14/group) adolescent rats. All groups of male (a) and female (b) subjects began consuming the palatable food ~300-400 seconds into the session, with no statistical differences among groups. Groups also did not differ in the total amount of food consumed for males (c) and females (d), although high within-group variability was noted. s = seconds, g = grams *p < .05 and **p < .01. Data represent mean ± SEM.

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Declaration of Interests

None declared.

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Data Availability

The data that support the findings of this study will be openly available at OSF (https://osf.io/dashboard) DOI 10.17605/OSF.IO/34H6P, upon manuscript acceptance.

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