Nicotine & Tobacco Research, 2020, 1364–1373
doi:10.1093/ntr/ntaa084
Original Investigation
Received October 6, 2019; Editorial Decision May 1, 2020; Accepted May 6, 2020

Original Investigation

Adolescent Cannabinoid and Nicotine Exposure Differentially Alters Adult Nicotine Self-Administration in Males and Females

Angeline J. Dukes BA, James P. Fowler, Valeria Lallai PhD, Anna N. Pushkin BS, Christie D. Fowler PhD

Department of Neurobiology and Behavior, University of California, Irvine, Irvine, CA

Corresponding Author: Christie D. Fowler, PhD, Department of Neurobiology and Behavior, University of California, Irvine, 1232 McGaugh Hall, Irvine, CA 92697, USA. Telephone: 949-824-8363; Fax: 949-824-2447; E-mail: cdfowler@uci.edu

Abstract

Introduction: During adolescence, exposure to nicotine or cannabis independently induces effects on neuromaturation and later cognitive function. However, the potential effect of both drugs under co-use conditions has become of increasing concern given the prevalence of e-cigarettes, legalization of cannabis, and availability of synthetic "spice" cannabinoid agonists.

Aims and Methods: The current studies investigated the effects of exposure to a cannabinoid receptor agonist (WIN55,212-2) and/or nicotine over a discrete time period in mid-adolescence on later intravenous nicotine self-administration in adult male and female mice. We further examined whether cannabinoid agonist administration in adulthood would alter nicotine reinforcement, with either acute or chronic pairing across 7 days.

Results: We found that adult males exhibited increased nicotine self-administration at a lower, rewarding nicotine dose following adolescent cannabinoid exposure, either alone or with nicotine coadministration. In contrast, adult females demonstrated an opposing effect in which adolescent cannabinoid and nicotine coexposure resulted in decreased nicotine intake compared with the nicotine only and control groups. Furthermore, after maintaining nicotine self-administration across sessions, pretreatment with a low dose of the cannabinoid agonist decreased nicotine intake in both male and female control mice, and this lowering effect was evidenced after both acute and chronic treatment. However, the cannabinoid agonist was ineffective in altering nicotine intake in mice previously exposed to nicotine, cannabinoid agonist, or both during adolescence.

Conclusions: These data provide evidence that adolescent drug exposure can alter later nicotine reinforcement in a sex-specific manner and can further modulate the effectiveness of interventions in reducing nicotine intake during adulthood.

Implications: These studies demonstrate a significant impact of nicotine, cannabinoids, or coexposure on developmental processes during adolescence. Differential effects were observed within each sex, with opposing results found for cannabinoid exposure on nicotine intake in males and females. Intriguingly, we also evidenced resistance to the lowering effects of a cannabinoid agonist on nicotine intake in adulthood based on adolescent drug exposure. Thus, these findings have important implications for our understanding of the impact of nicotine and cannabinoids (eg, Δ^9-tetrahydrocannabinol (THC) and synthetic "spice" cannabinoids) during development, with further implications for the effectiveness of therapeutic interventions based on prior drug exposure in youth.

Introduction

Nicotine dependence is among the largest preventable causes of disease and death worldwide. Further, polydrug use, including that of nicotine and cannabis, may lead to interactive effects on brain neurocircuitries.[1] Thus, this study represents the first to begin deciphering the coconsumption effects of nicotine and cannabinoids during adolescent development on later dependence and/or resistance to achieving abstinence. According to a 2015 nationwide survey, 32.3% of high school students self-reported prior cigarette use, whereas 44.9% reported using vaporized nicotine products.[2] Of further concern, 38.6% of these students reported using cannabis.[2] Given that recreational cannabis use was illegal in most states at the time of this survey, the number of adolescents exposed to this drug will likely only increase through both primary use and secondhand exposure as the drug becomes more readily accessible. This is supported by the finding that 44% of 12th graders in a recent 2018 nationwide survey reported using cannabis in their lifetime.[3] Further, the practice of mulling, combining tobacco with cannabis to smoke as a joint, has been reported as frequently occurring in adolescent users, with highest incidence (up to 90%) among daily cigarette smokers in some populations.[4,5] Furthermore, individuals who reported smoking cannabis and tobacco cigarettes consumed more cigarettes than those smoking cigarettes alone.[6] Together, these findings have introduced increasing concerns regarding the interaction between the drugs and the effects of early adolescent exposure on later drug-taking behaviors.

Nicotine, the main psychoactive component in tobacco and e-cigarettes, acts in the brain on neuronal nicotinic acetylcholine receptors (nAChRs), and the psychoactive effects of cannabis have been attributed to action on the cannabinoid 1 receptor (CB1R). The CB1Rs are also targeted by other abused drugs, such as synthetic "spice" cannabinoid agonists for which the majority belong to the aminoalkylindole class, including WIN55,212-2.[7–9] The nAChRs and CB1Rs exhibit overlapping expression patterns within brain regions implicated in drug reinforcement and aversion, including the prefrontal cortex, ventral tegmental area, nucleus accumbens, medial habenula, interpeduncular nucleus, and hippocampus.[10,11] On the cellular level, CB1Rs and nAChRs are expressed on presynaptic axon terminals, and both function to modulate release of neurotransmitters.[11,12] Reciprocal outcomes are found in their actions and behavioral effects. Exogenous cannabinoids can modulate cholinergic neurotransmission in the brain,[13] and similarly, nicotine administration alters endogenous cannabinoid signaling.[14] Further, similar effects are found with neurotransmitter release; for instance, administration of either nicotine or the CB1R agonist, WIN55,212-2, increases extracellular dopamine in the nucleus accumbens and prefrontal cortex.[15,16] These findings provide evidence to support the notion that exogenously derived cannabinoid or cholinergic modulation of neurotransmission during adolescence may lead to various altered drug-associated behaviors along the continuum of the dependence processes.

In humans, tobacco exposure during development has been associated with increased drug use during adulthood.[17,18] However, given the nature of human studies, it is unclear as to whether the early life exposure increases vulnerability, or whether a preexisting neural state and/or environmental factors prompted consumption of the drug products. In rodents, adolescent nicotine exposure results in increased time spent in an environment associated with nicotine during adulthood,[19] suggesting an enhanced rewarding effect of nicotine following prior exposure. In an oral self-administration study, rats that drank a nicotine solution during late adolescence into early adulthood (postnatal day [PND] 35–77) exhibited either a similar level or diminished nicotine drinking behavior in later adulthood (PND 140+).[20] However, high variability in the amount of nicotine consumed has been found in such oral drinking paradigms,[20] potentially due to activation of nAChRs expressed in the tongue and/or postconsummatory gastrointestinal effects. In contrast, the intravenous nicotine self-administration procedure is generally accepted as having greater translational relevance to human behavior, as stable responding and titration of intake are found across doses.[21,22] A few studies have examined adolescent nicotine exposure on later nicotine self-administration in adulthood. In one study in rats, nicotine exposure during PND 25–42 did not alter later nicotine self-administration behavior during early adulthood,[23] but it should be noted that the subjects in this study were individually housed and shipped during PND 20–21[23]; factors that could have elicited stressful conditions during the adolescent period. In contrast, another study found a decrease in the motivation to self-administer nicotine during adulthood; in this paradigm, subjects had variable access to a range of nicotine doses for self-administration, including high aversive doses, beginning at PND 34, and prior to adult testing,[24] which may have subsequently biased the resultant level pressing behavior.

Here, we sought to examine whether adolescent exposure to nicotine and/or a cannabinoid agonist would alter intravenous nicotine self-administration during adulthood in male and female mice. The current investigations focus on the coexposure condition, which is commonly found in human subjects, and the resulting effects on later nicotine intake. Adolescent mice were exposed to a moderate or low dose of the cannabinoid receptor agonist, WIN55,212-2, and/or nicotine and then were assessed for nicotine reinforcement behaviors during adulthood. Drug exposure occurred during PND 38–49, which corresponds to mid-adolescence in rodents or ~13–17 years of age in humans.[25,26] Given the previously established differential responses for males and females with drug-related effects and baseline receptor expression across development,[11,25,27,28] male and female mice were examined in a within-sex manner. Finally, we also examined whether acute or repeated administration of the cannabinoid agonist during adulthood would alter nicotine self-administration dependent on the prior adolescent exposure condition. The goal of this study was to determine if an interaction effect would occur during adulthood, in consideration of each adolescent exposure condition. Together, these studies provide evidence that adolescent drug exposure alters nicotine reinforcement in a sex-dependent manner and prevents the dampening effects of a cannabinoid on nicotine intake during adulthood in both sexes.

Methods

Animals

Male and female wild-type C57BL/6J mice were derived from breeders in our laboratory animal facilities; in total, 54 male and 63 female mice were examined in these studies. Mice were maintained in an environmentally controlled vivarium on a 12-hour reversed light/dark cycle. Food and water were provided *ad libitum* until behavioral training commenced. During food and nicotine self-administration, subjects were mildly food restricted to 85%–90% of their free-feeding body weight, and water was provided *ad libitum*. All experiments were conducted in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and were

approved by the Institutional Animal Care and Use Committee at the University of California, Irvine.

Drugs

The cannabinoid receptor agonist WIN55,212-2 mesylate (Tocris/Bio-Techne Corp, Minneapolis, ME) was dissolved in vehicle containing 1% dimethyl sulfoxide, 1% Tween-80, and 98% saline (sterile 0.9% NaCl). The doses of WIN55,212-2 administered were 0.2 or 2 mg/kg, intraperitoneally (i.p.). The moderate dose of WIN (2 mg/kg) was selected based on prior studies demonstrating altered neural function with adolescent exposure in mice and rats,[29,30] and the low dose of WIN (0.2 mg/kg) was selected based on evidence from adolescent WIN self-administration in rats (~16 infusions/day at 0.0125 mg/kg/infusion = ~0.2 mg/kg/day).[31] (−)-Nicotine hydrogen tartrate salt (MP Biomedicals, Santa Ana, CA; 0215355491) was dissolved in 0.9% sterile saline and adjusted to pH 7.4. Nicotine was administered at a dose of 0.36 mg/kg, subcutaneous (s.c.) (free-base form); this dose is considered to be within the rewarding range of the dose–response function that also elicits a behavioral response in adolescent C57BL/6J mice.[28,32,33] Peripheral injections were administered at a volume of 10 mL/kg.

Adolescent Injection Schedule

Beginning on PND 38, the first set of male and female mice were randomly subdivided into four experimental groups: (1) Control (saline s.c., vehicle i.p.), (2) NIC (0.36 mg/kg nicotine s.c., vehicle i.p.), (3) WIN (saline s.c., 2 mg/kg WIN i.p.), and (4) NIC/WIN (0.36 mg/kg nicotine s.c., 2 mg/kg WIN i.p.). Saline and vehicle were the solutions used to dissolve nicotine and WIN, respectively. Mice received once daily injections for 12 consecutive days from PND 38 to PND 49. This timeframe is considered mid-adolescence in rodents, corresponding to ~13–17 in human years.[26] This represents a dynamic developmental period for both the endogenous nicotinic acetylcholine and cannabinoid systems; for instance, the highest level of CB1 receptor expression is found during this period.[11,25,27,28,34] The daily injection schedule was selected to model an experimental pattern of adolescent exposure as previously described.[28] Body weight was recorded prior to each injection. The second set of male and female mice were treated as above, but they were subdivided into the following experimental groups: (1) Control (saline s.c., vehicle i.p.), (2) LdWIN (saline s.c., low dose [0.2 mg/kg] WIN i.p.), and (3) NIC/LdWIN (0.36 mg/kg nicotine s.c., 0.2 mg/kg WIN i.p.). All above groups were tested in multiple smaller cohorts to enhance rigor and reproducibility of the findings. The current studies were designed to systematically assess changes following adolescent exposure under the varying conditions by maintaining precise dosing conditions via peripheral injections.

Intravenous Nicotine Self-Administration

Mice were mildly food restricted to 85%–90% of their free-feeding body weight and trained to press a lever in an operant chamber (Med Associates, St. Albans, VT) for food pellets (20 mg; TestDiet, Richmond, IN) under a fixed-ratio 5, time out 20 seconds (FR5TO20 sec) schedule of reinforcement. We have previously shown that these adolescent exposure groups do not differ in operant food learning.[28] Once stable responding was achieved (>25 pellets per session across three subsequent sessions), subjects were surgically catheterized as previously described.[21,35] Briefly, mice were anesthetized with an isoflurane (1%–3%)/oxygen vapor mixture and prepared with intravenous catheters. Catheters consisted of a 6 cm length of silastic tubing fitted to guide cannula (Plastics One, Roanoke, VA) bent at a curved right angle and encased in dental acrylic. The catheter tubing was passed subcutaneously from the animal's back to the right jugular vein, and a 1 cm length of the catheter tip was inserted into the vein and tied with surgical silk suture. Following the surgical procedure, animals were allowed ≥48 hours to recover from surgery, then provided access to again respond for food reward. Mice were then permitted to acquire intravenous nicotine self-administration during 1 hour daily sessions, 6–7 days per week, at the standard training dose of nicotine (0.03 mg/kg/infusion). Nicotine was delivered through tubing into the intravenous catheter by a Razel syringe pump (Med Associates). Each session was performed using two retractable levers (one active, one inactive). Completion of the response criteria on the active lever resulted in the delivery of an intravenous nicotine infusion (0.03 mL infusion volume; FR5TO20 sec schedule). Responses on the inactive lever were recorded but had no scheduled consequences. Catheters were flushed daily with physiological sterile saline solution (0.9%, w/v) containing heparin (10 USP units). Catheter integrity was tested with the short-acting barbiturate anesthetic Brevital (methohexital sodium, Eli Lilly, Indianapolis, IN). Subjects and their data were removed from the study due to death or if the catheter integrity was compromised as determined by visual leakage or Brevital assessment. Behavioral responses were automatically recorded by MedAssociates software.

Experimental Design

The experimental design is outlined in Figure 1. Following adolescent injections, mice remained drug-free until adulthood (PND 70). Thereafter, they were examined for differences in cognitive behavior as reported previously.[28] For these investigations, to ascertain the dose–response function, mice were tested according to the established mouse intravenous self-administration protocol.[21] Following an acquisition period of at least 7 days on the training dose (0.03 mg/kg/infusion), the animals were presented with a different dose of nicotine for at least 5 days, and the mean intake for the last two sessions was used for statistical analyses. In between each dose, subjects were returned to the 0.1 mg/kg/infusion dose for 2 days or until intake returned to baseline levels. The dose–response function occurred over a total ~35 sessions with testing sessions occurring 6 days per week. Thereafter, mice were stabilized on the moderate 0.1 mg/kg/infusion dose across three baseline sessions after successfully passing the Brevital catheter patency test. Then, subjects were challenged with an injection of the low dose WIN (0.2 mg/kg) or vehicle control, 20 minutes prior to the nicotine self-administration session. Injections of vehicle or low dose WIN were administered in a random, counterbalanced design both within and across groups, and subjects were permitted at least 2 baseline days in between WIN/vehicle administration to return to baseline levels of nicotine intake. After the crossover experiment with the single, acute dose of WIN, mice were chronically pretreated with the same low WIN dose prior to each session across seven consecutive sessions, and nicotine intake on the seventh session was used to determine the effects of chronic coexposure during adulthood for all groups. Since the control groups (adolescent vehicle treatment) for the moderate and low dose WIN cohorts exhibited similar effects with pretreatment, data were compiled into one graph for each sex. Finally, mice were again returned to self-administer the 0.1 mg/kg/infusion dose, and after achieving baseline levels of responding, they were then transitioned to respond for saline infusions (no nicotine). Eleven mice were required to be

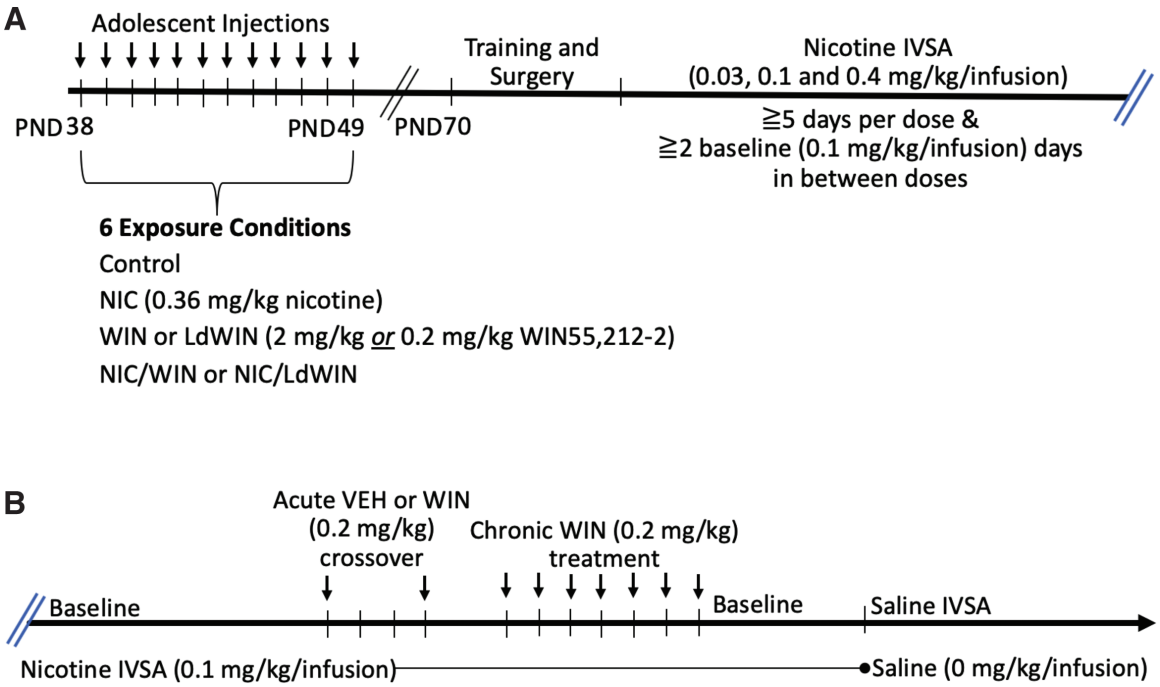

Figure 1. Schematic outline of the experimental design. (A) All mice were treated in adolescence with nicotine (NIC), WIN55,212-2 (WIN), NIC/WIN coexposure, or vehicle (VEH) control. WIN was administered at either a low or high dose for the single and coexposure conditions. After PND 70, mice began testing for subsequent examination of intravenous nicotine self-administration (IVSA) across doses. (B) After reestablishing baseline responding on the 0.1 mg/kg/infusion nicotine dose, mice were then pretreated across sessions with vehicle or low dose WIN in a crossover design. Thereafter, mice were examined with chronic low dose WIN and nicotine self-administration coexposure for seven consecutive testing sessions. Finally, after again reestablishing baseline responding, mice were transitioned to respond for saline infusions in the absence of nicotine. PND = postnatal day.

excluded due to death/cannibalization by cagemates (three female Control, one female NIC, three female and one male NIC/WIN, two female NIC/LdWIN, and one male LdWIN), and six were excluded due to compromised catheter integrity (two female Control, two female NIC, one female NIC/LdWIN, and one male LdWIN).

Statistical Analyses

Given that these studies sought to investigate the effects of drug exposure relative to the control condition within each sex, statistical comparisons were performed separately for males and females based on this a priori hypothesis.[28] Data were analyzed by a *t* test, one-way or two-way analysis of variance (ANOVA) with Prism 7 software (GraphPad, La Jolla, CA), as appropriate. Data obtained across sessions were analyzed with a repeated measures two-way ANOVA. Significant main or interaction effects were followed by Bonferroni post hoc comparison with correction for multiple comparisons. The criterion for significance was set at $\alpha = 0.05$.

Results

Intravenous Nicotine Self-Administration During Adulthood

Adolescent exposure groups were examined for differences in nicotine intake during adulthood across low, moderate, and high self-administration doses (Figure 1A). This approach allows for the assessment of the dose–response function, which provides a measure of responding across nicotine doses with increasing value of reinforcement (ascending limb of the dose–response) and doses inducing greater aversion and/or satiation (descending limb of the dose–response).[21] In male mice, significant differences were found on the ascending limb at the 0.03 mg/kg/infusion dose, but not at higher doses (Figure 2A) (repeated measures two-way ANOVA, Group $F_{(3,25)} = 2.13$, $p = .122$; Dose $F_{(3,75)} = 38.15$, $p < .0001$; Interaction $F_{(9,75)} = 2.29$, $p = .024$). Specifically, the WIN and nicotine/WIN adolescent exposure groups exhibited a significantly increased number of nicotine infusions compared with the control and nicotine adolescent exposure groups ($p < .05$ for WIN compared with either control or nicotine; $p < .01$ for nicotine/WIN compared with either control or nicotine). Further, the groups did not differ in their saline level of responding, indicating that these differences were not due to a general increase in lever pressing behavior. Since both the WIN and nicotine/WIN exposure conditions involved a moderate dose of the cannabinoid agonist (2 mg/kg), we next addressed the possibility that this WIN dose could have masked the effects of nicotine in an interactive effect. Thus, we examined a separate cohort of mice exposed to a low dose of WIN (0.2 mg/kg), either in the presence or absence of nicotine. However, differences were not found in the dose–response function among these adolescent treatment conditions, with all groups exhibiting a main effect for nicotine dose (Figure 2B) (repeated measures two-way ANOVA, Group $F_{(2,19)} = 1.06$, $p = .368$; Dose $F_{(3,57)} = 15.51$, $p < .0001$; Interaction $F_{(6,57)} = 0.845$, $p = .541$).

In female mice, statistically significant main and interaction effects were found among the control, nicotine, WIN, and coexposure nicotine and WIN groups (Figure 2C) (repeated measures two-way ANOVA, Group $F_{(3,24)} = 5.24$, $p = .006$; Dose $F_{(3,72)} = 33.44$, $p < .0001$; Interaction $F_{(9,72)} = 2.82$, $p = .007$). The post hoc analysis revealed an upward shift in the dose–response function for the nicotine exposure group, as compared with both the WIN and coexposure nicotine and WIN groups. Specifically, at the 0.03 mg/kg/infusion

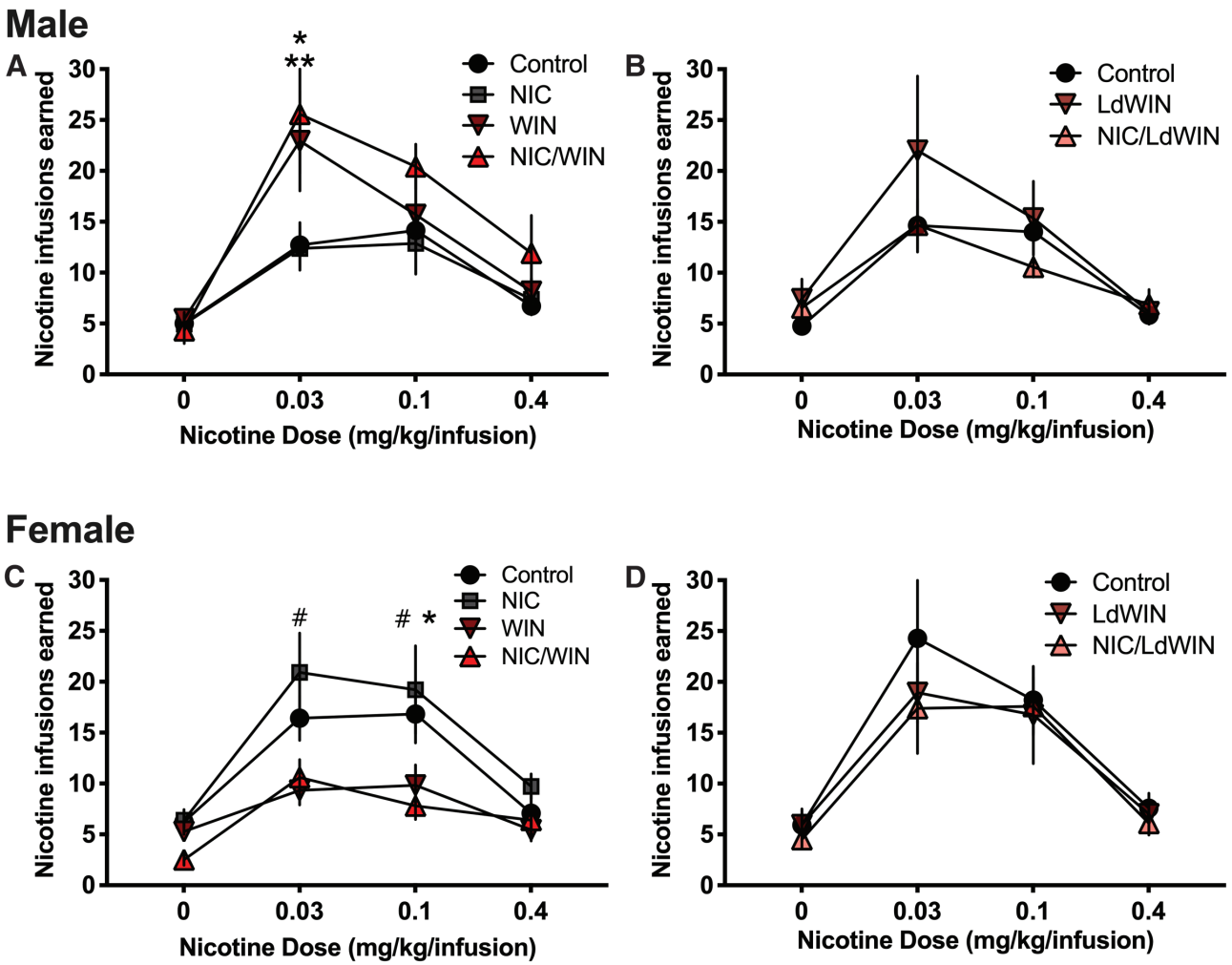

Figure 2. Male and female mice exposed to the cannabinoid agonist during adolescence exhibit opposing effects on nicotine self-administration in adulthood. (A and B) Male intravenous nicotine self-administration dose–response function. (A) Following exposure to the cannabinoid agonist WIN (2 mg/kg) during adolescence, adult male mice demonstrated an increase in nicotine intake on the ascending limb of the dose–response function at the 0.03 mg/kg/infusion dose compared with the vehicle control and nicotine only (NIC) groups. A similar increase in nicotine intake was also found with nicotine and WIN coexposure (NIC/WIN) at this dose compared with both the vehicle and NIC groups (n = 6–8/group). *p < .05 Control vs. WIN, and NIC vs. WIN; **p < .01 Control vs. NIC/WIN, and NIC vs. NIC/WIN. (B) Adult male mice administered the lower dose of WIN (0.2 mg/kg), either in the presence or absence of nicotine, during adolescence did not exhibit statistically significant differences from the control across the dose–response function (n = 7–8/group). (C and D) Female intravenous nicotine self-administration dose–response function. (C) Adult female mice exposed to nicotine (NIC) during adolescence earned significantly more nicotine infusions at the low (0.03 mg/kg/infusion) and moderate (0.1 mg/kg/infusion) doses, as compared with the adolescent-exposed cannabinoid agonist WIN (2 mg/kg) groups, either alone or with nicotine coexposure. The coexposure WIN and nicotine group also earned significantly less nicotine infusions than the control condition at the moderate 0.1 mg/kg/infusion dose (n = 6–9/group). #p < .01 NIC vs. WIN or NIC/WIN; *p < .05 Control vs. NIC/WIN. (D) Adult female mice administered the low dose of WIN during adolescence, alone or with nicotine, did not differ from the control vehicle group across the dose–response function (n = 6–8/group). Data represent mean values ± standard error of the mean (SEM).

dose, the adolescent nicotine group exhibited a significantly greater number of nicotine infusions than the adolescent WIN (p < .001) and nicotine/WIN coexposure (p < .01) groups. At the moderate 0.1 mg/kg/infusion dose, the nicotine group also demonstrated a statistically significant increase from the WIN group (p < .01) and nicotine/WIN coexposure group (p < .001), and the nicotine/WIN coexposure group was also significantly decreased compared with the control group (p < .05). No other groups significantly differed from the control, or at the saline and high dose of nicotine (0.4 mg/kg/infusion). Thereafter, a second set of female mice were examined for differences with the lower dose of WIN. However, the low dose WIN adolescent exposure groups, either in the presence or absence of nicotine, did not differ across the dose–response function from the control condition, with a significant main effect of dose evidenced (Figure 2D) (repeated measures two-way ANOVA, Group $F_{(2,18)}$ = 0.42, p = .662; Dose $F_{(3,54)}$ = 29.26, p < .0001; Interaction $F_{(6,54)}$ = 0.45, p = .842).

Interactive Effects of Acute or Chronic WIN Exposure During Adult Nicotine Self-Administration

To examine whether further exposure in adulthood to a cannabinoid agonist subsequently alters nicotine intake, mice were pretreated with the low dose of the cannabinoid agonist or vehicle prior to a nicotine self-administration session; thereafter, the mice were then repeatedly administered the low dose of the cannabinoid agonist prior to seven

consecutive nicotine self-administration sessions (Figure 1B). In adult males, we found that both acute and chronic treatment with WIN significantly attenuated nicotine intake relative to the vehicle control (repeated measures one-way ANOVA, $F_{(2,32)} = 8.09$, $p = .001$; Post hoc, vehicle vs. acute $p < .05$, vehicle vs. chronic $p < .01$) (Figure 3A), indicating that cannabinoid co-use in adulthood reduces nicotine consumption. Interestingly, when we examined the adolescent-exposed nicotine and WIN groups, a stark contrast in responding was evidenced. Across all adolescent drug groups, the cannabinoid agonist was ineffective in altering nicotine intake relative to infusions earned following vehicle injection (repeated measures one-way ANOVAs: Nicotine, $F_{(2,14)} = 0.37$, $p = .695$; WIN, $F_{(2,16)} = 0.61$, $p = .554$; Low dose WIN, $F_{(2,12)} = 5.77$, $p = .018$; Nicotine and WIN coexposure, $F_{(2,8)} = 1.75$, $p = .234$; Nicotine and low dose WIN coexposure, $F_{(2,14)} = 2.67$, $p = .104$) (Figure 3B, C, D, E, and F, respectively).

In adult females, the control group exhibited a similar effect of cannabinoid agonist pretreatment in reducing nicotine intake as to that found in the males (repeated measures one-way ANOVA, $F_{(2,26)} = 15.94$, $p < .0001$) (Figure 4A). Specifically, in post hoc analyses, the vehicle condition exhibited a higher level of nicotine infusions compared with pretreatment with the cannabinoid after one session (acute, $p < .05$) and after seven consecutive sessions (chronic, $p < .0001$). Further, chronic administration of the cannabinoid agonist significantly reduced nicotine intake to a greater extent than the acute condition ($p < .05$). However, adolescent drug exposure resulted in a resilience to the effects of the cannabinoid agonist during adulthood on nicotine intake, since differences were not found in the number of nicotine infusions earned after cannabinoid agonist injection for all other groups (repeated measures one-way ANOVAs: Nicotine, $F_{(2,12)} = 3.09$, $p = .083$; WIN, $F_{(2,16)} = 2.16$, $p = .148$; Low dose WIN, $F_{(2,14)} = 2.11$, $p = .158$; Nicotine and WIN coexposure, $F_{(2,10)} = 2.11$, $p = .173$; Nicotine and low dose WIN coexposure, $F_{(2,10)} = 0.27$, $p = .771$) (Figure 4B, C, D, E, and F, respectively).

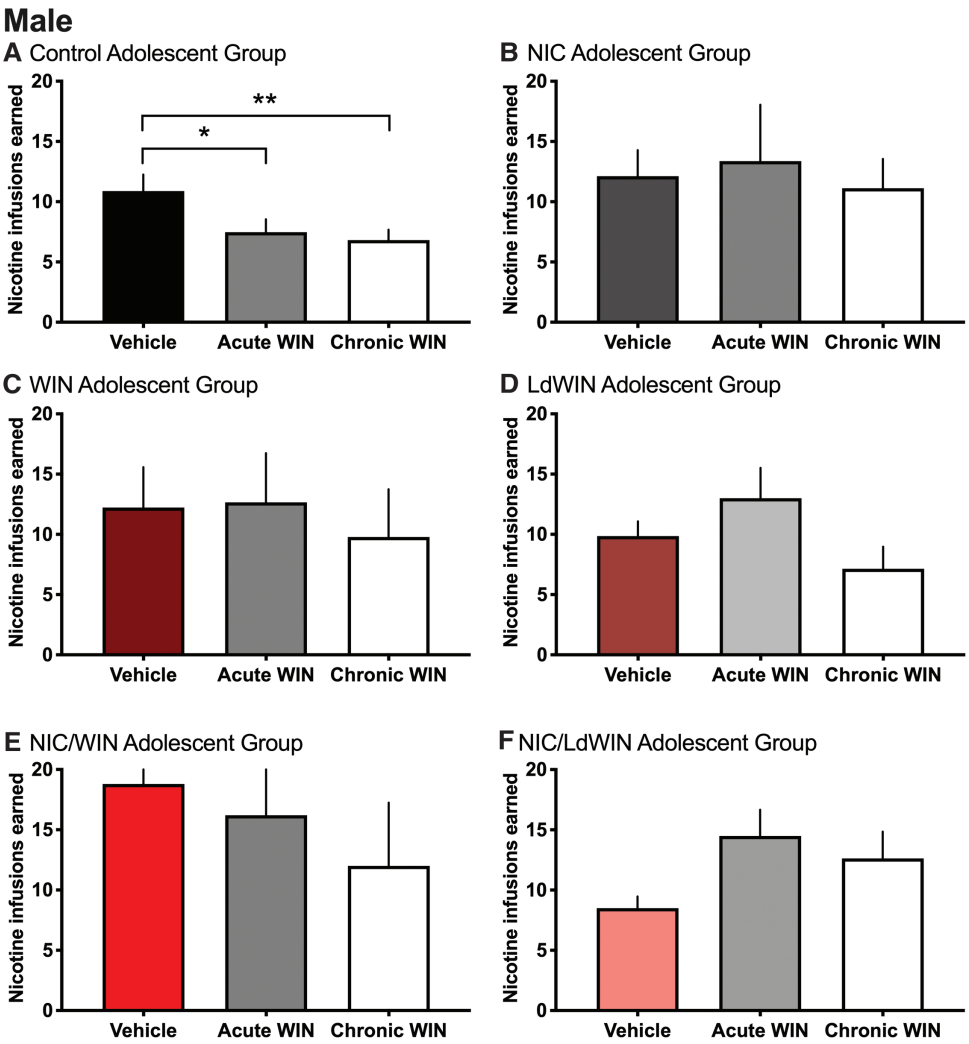

Figure 3. Adolescent drug exposure in male mice results in resistance to the effects of the cannabinoid agonist on nicotine intake in adulthood. (A) Control male mice exhibit a statistically significant reduction in nicotine intake after acute cannabinoid agonist preadministration and following 7 consecutive days of treatment (chronic) in adulthood ($n = 17$). *$p < .05$, **$p < .01$ compared with vehicle injection. (B–F) Administration of a low dose of the cannabinoid agonist during adulthood was ineffective in altering nicotine intake in male mice exposed to the following during adolescence: (B) nicotine ($n = 8$), (C) the cannabinoid agonist WIN ($n = 9$), (D) low dose of the cannabinoid agonist WIN ($n = 7$), (E) coexposure of nicotine and the cannabinoid agonist WIN ($n = 5$), or (F) coexposure of nicotine and the low dose of the cannabinoid agonist WIN ($n = 8$). Data represent mean values ± standard error of the mean (SEM).

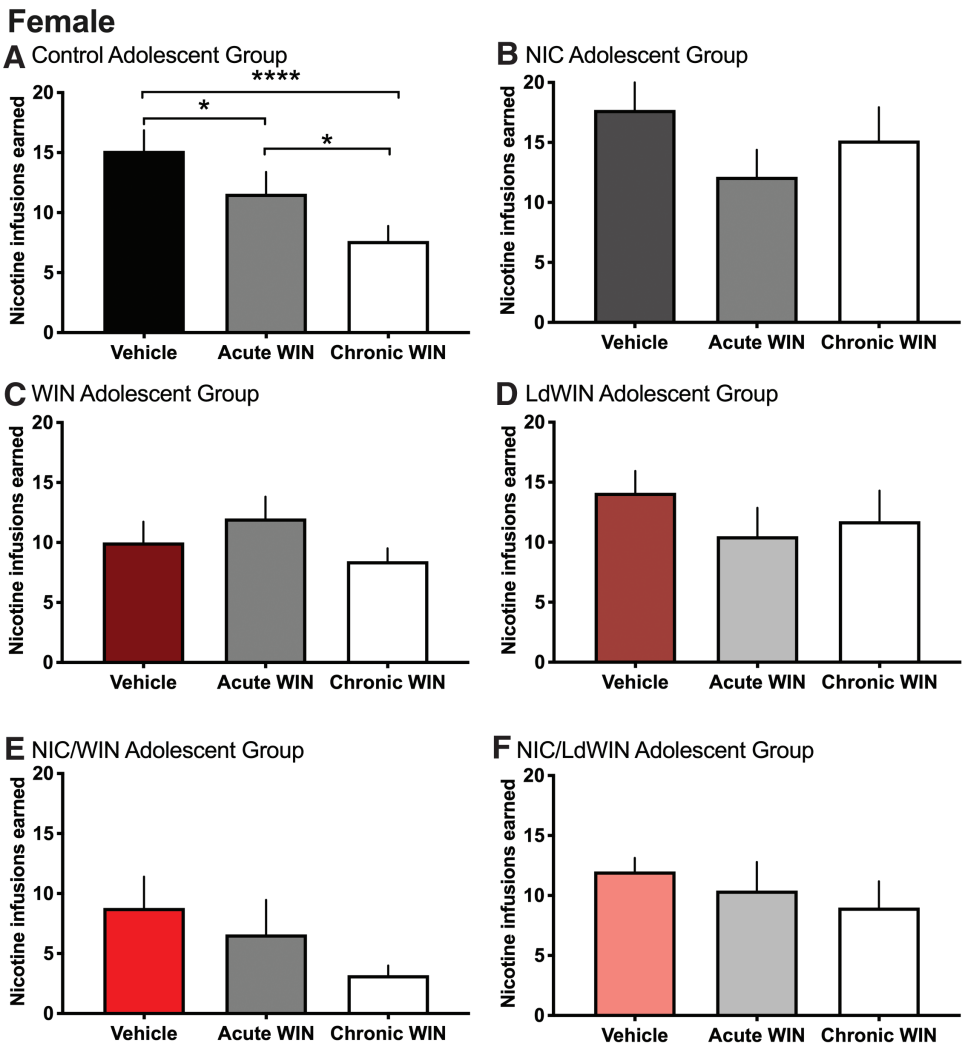

Figure 4. Adolescent nicotine or cannabinoid agonist exposure in female mice prevents the lowering effect of the cannabinoid agonist on nicotine intake in adulthood. (A) Control female mice earned significantly fewer nicotine infusions both after an acute cannabinoid agonist preadministration and with chronic exposure ($n = 14$). *$p < .05$ for vehicle versus acute WIN, and acute WIN versus chronic WIN, ****$p < .0001$ for vehicle versus chronic WIN. (B–F) The cannabinoid agonist was ineffective in altering nicotine intake during adulthood in female mice with adolescent exposure to (B) nicotine ($n = 7$), (C) the cannabinoid agonist WIN ($n = 9$), (D) low dose of the cannabinoid agonist WIN ($n = 8$), (E) coexposure of nicotine and the cannabinoid agonist WIN ($n = 6$), or (F) coexposure of nicotine and the low dose of the cannabinoid agonist WIN ($n = 6$). Data represent mean values ± standard error of the mean (SEM).

Discussion

In these studies, we found that adolescent cannabinoid and/or nicotine exposure exert a lasting impact on susceptibility to drug reinforcement, which is evidenced in adulthood. However, these effects were dependent on the substance of abuse (cannabinoid agonist or nicotine), dose of the cannabinoid, and sex. Specifically, adult males exhibited increased nicotine self-administration at the lower rewarding nicotine dose following adolescent cannabinoid agonist exposure at the moderate dose (2 mg/kg), either alone or with nicotine coadministration. In contrast, adult females demonstrated an opposing effect following adolescent cannabinoid exposure at the moderate dose, in which such exposure resulted in decreased nicotine intake compared with nicotine exposure alone. However, differences were not induced within either sex with adolescent exposure to the lower dose of the cannabinoid agonist (0.2 mg/kg). Furthermore, after maintaining nicotine self-administration, pretreatment with the low dose of the cannabinoid agonist attenuated nicotine intake in both male and female control mice, and this lowering effect was evidenced both acutely and after chronic pairings. Surprisingly, the cannabinoid agonist was ineffective in altering nicotine intake in mice previously exposed to nicotine, the cannabinoid agonist, or both during adolescence; an effect that was present at both the lower and moderate doses of the cannabinoid agonist.

Impact of Adolescent Drug Exposure on Adult Nicotine Intake

Nicotine self-administration produces an inverted U-shaped dose–response curve, which represents the competing positive and negative properties of nicotine. The increased responding for nicotine over the ascending limb of the curve reflects the increasing reinforcing effects of nicotine as the unit dose increases. In contrast, the decreased responding over the descending limb of the curve reflects the increasing aversive properties of nicotine or satiation. Mesolimbic dopamine neurons have been primarily implicated in modulating

the rewarding and reinforcing aspects of the drug,[36] whereas the aversive signaling of nicotine appears to involve the habenulo-interpeduncular pathway.[12] Our findings suggest that adolescent cannabinoid exposure most likely alters the function of the mesolimbic pathway, as differences were found primarily on the ascending limb of the dose–response function. In support of this notion, adolescent cannabinoid or nicotine exposure has previously been shown to alter monoaminergic signaling.[37–40] However, in our study, nicotine alone was ineffective in altering later drug-taking behaviors in males, either in combination with the cannabinoid or alone. Since studies have shown that of those adolescents age 12–17 who smoke, the majority smoke one or less than one cigarette per day (50.1%),[41] the current studies focused on a rewarding dose with once daily exposure of a rewarding dose.[28,32,33] Thus, the current results have particular relevance to experimental patterns of drug consumption found in youth.

Differential patterns of expression of the cannabinoid receptors are found across adolescent development and between males and females,[42] and CB1Rs exhibit highest level of expression during the developmental period of mid-adolescence (PND 25–50).[42] Thus, the potential for exogenous cannabinoids to alter synaptic and neural circuit function may be considered greatest during this time period. Indeed, prior studies have revealed an effect of CB1R activation on adolescent brain development and indicate a correlation between adolescent exposure and later cognition and reward-related function. For instance, we found that adult males exposed during adolescence to the moderate 2 mg/kg dose of WIN exhibited increased cognitive flexibility in a learning reversal task, decreased anxiety-associated behaviors, and increased natural reward consumption with the same exposure paradigm.[28] The coexposure condition of both nicotine and the moderate dose of WIN also led to similar behavioral profiles as WIN alone in these measures,[28] suggesting that a potentiative or additive effect was not present similar to that found in the current studies with nicotine intake. With regard to females, they were found to be overall more resistant to the long-term effects of adolescent drug exposure, in which the moderate dose WIN females exhibited decreased natural reward consumption compared with the control females.[28] Interestingly, CB1R knockout mice are resistant to nicotine-mediated locomotion and conditioned place preference, but do not differ in nicotine self-administration, as compared with wild-type mice,[43,44] which suggests that the lack of CB1Rs during adulthood may affect generalized locomotor behavior and drug-conditioned memory function, but perhaps not the motivation to consume nicotine. However, given the constitutive knockout of the gene in these mice, it is possible that compensatory mechanisms occurred during development, resulting in altered expression of other receptors, potentially including cannabinoid 2 receptors and/or nAChRs.

Adolescent Exposure Infers Resistance to a Cannabinoid-Induced Decrease in Nicotine Intake

Both single and co-use of nicotine and cannabinoid products are prevalent during adolescence and adulthood. Thus, we further examined coexposure during adulthood, under both control conditions and following adolescent drug exposure. In the control group, we found that the low dose of the cannabinoid agonist reduced nicotine intake in adulthood. This represents the first demonstration of the effects of a cannabinoid agonist on intravenous nicotine self-administration in mice. These results were surprising since the CB1 receptor antagonists rimonabant and taranabant have also been shown to reduce nicotine consumption.[45] However, when one considers that additive effects may be induced on brain reward circuitries, such as that found with reduced brain stimulation thresholds in the presence of rewarding doses of nicotine, it is likely that the presence of the cannabinoid agonist augmented the activity of the reward circuits in the brain, leading to a reduction in nicotine intake while maintaining similar circuit activation to support drug reinforcement. However, this stipulation will need to be more directly tested in future studies.

We further found that the effectiveness of the cannabinoid agonist in reducing nicotine self-administration is dependent on prior drug exposure during adolescence, as all of the adolescent nicotine and/or WIN exposure groups did not differ in nicotine intake with WIN pretreatment in adulthood. Of further note, we found that this lack of responsiveness to the dampening effects of the cannabinoid agonist on nicotine intake also occurred in adolescent groups exposed to the low dose of the cannabinoid agonist. It is important to note that this level of exposure did not induce any other detectable behavioral effects during adulthood, either in this study or in our prior analysis of cognitive, anxiety-, and depression-associated behaviors.[28] Given these findings, it is possible that patients may differentially respond to pharmacotherapeutics based on developmental drug exposure, representing a potential underlying factor mitigating individual differences in cessation outcomes. Indeed, given that we found differences in nicotine intake during adulthood with developmental drug exposure, and currently available pharmacotherapeutics such as varenicline also target nAChRs, similar signaling mechanisms may be involved in mitigating the behavioral responses to these drug compounds.

Finally, in these studies, we examined the effects of an injected cannabinoid agonist during adolescent development on nicotine self-administration in adulthood. Importantly, these results have direct implications for the use of "spice" synthetic cannabinoids, of which the majority belong to the aminoalkylindole class, including WIN55,212-2.[7–9] In addition, these findings likely have further implications for cannabis exposure. Δ^9-Tetrahydrocannabinol (THC) has been characterized as a partial agonist of the CB1R, and therefore, it is possible that the low dose of the WIN agonist could have resulted in the occupation of a fewer number of receptors, thereby inducing an effect more similar to a higher dose of a partial agonist on downstream cellular signaling. However, this will need to be more systematically addressed in future studies. Moreover, while it is possible that volitional intake during adolescence may differentially alter drug reinforcement, rather than experimenter administered injections, there are some caveats to such an experimental design. First, it is not yet feasible to implant intravenous catheters in adolescent mice. Second, the dose that each animal receives cannot be discretely controlled with self-administration studies. This point is further compounded by the fact that coexposure conditions result in different intake amounts of each drug, as compared with single use conditions. Furthermore, both THC and WIN self-administration in rodent models have been difficult to establish in many labs, although some have been successful due to specific doses and reinforcement testing paradigms.[31,46,47] In particular, for THC in rats, the combined presence of cannabidiol appears to be necessary to enhance the development of sustained self-administration behavior in both intravenous and vapor paradigms.[47,48] This is interesting given that many THC e-cigarette vapes on the market do not contain cannabidiol, at least as indicated on commercial packaging. Given these considerations and with the foundational findings derived from the current studies, it will nevertheless be important in future studies to develop models for volitional adolescent nicotine and cannabinoid

self-administration, perhaps via vapor exposure, and then to determine whether the variable, self-titrated levels of each drug differentially impacts nicotine and/or cannabinoid self-administration in adulthood.

Conclusions

In these studies, we have found that adolescent cannabinoid and/or nicotine exposure leads to differential effects on nicotine-taking behaviors in male and female mice. Further, such developmental exposure appears to alter the brain's later responsiveness with important implications for co-use conditions, in which developmental cannabinoid or nicotine exposure leads to sustained use of nicotine with cannabinoid coexposure in adulthood. In future studies, it will be important to examine both self-administered nicotine and cannabinoid exposure during adolescence and throughout the transition from adolescence to adulthood, as well as adolescent nicotine and cannabinoid exposure on other aspects of nicotine dependence, including withdrawal and relapse-related reinstatement behaviors. It will also be important to assess whether the impact of adolescent exposure differs due to genetic factors mitigating vulnerability. For instance, it has been demonstrated that humans with allelic variation in the catechol-O-methyltransferase gene are more likely to develop schizophrenia-related symptomology following adolescent cannabinoid use,[49] a finding that is of further relevance given the very high comorbidity found between schizophrenia and nicotine dependence.[50] In sum, given the increased adolescent use of nicotine and THC containing e-cigarettes, along with the availability of cannabis and synthetic "spice" products, the long-term consequences of developmental drug exposure represent an important health issue, and as such, the current findings should serve to guide future policy efforts to limit youth exposure.

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.

Funding

This research was supported by the Tobacco and Related Disease Research Program (TRDRP) award 26IP-0043 (CDF), the National Science Foundation Graduate Research Fellowship (NSF GRFP) award DGE-1839285 (AJD), National Institutes of Health (NIH) National Institute on Drug Abuse (NIDA) grant DA032543 (CDF), and University of California Smoke and Tobacco Free Fellowship (ANP).

Declaration of Interests

None declared.

References

1. Filbey FM, Gohel S, Prashad S, Biswal BB. Differential associations of combined vs. isolated cannabis and nicotine on brain resting state networks. *Brain Struct Funct*. 2018;223(7):3317–3326.
2. Kann L, McManus T, Harris WA, et al. Youth risk behavior surveillance—United States, 2015. *MMWR Surveill Summ*. 2016;65(6):1–174.
3. Miech R, Johnston LD, O'Malley PM, Bachman JG, Schulenburg JE, Patrick ME. *Monitoring the Future National Survey Results on Drug Use, 1975–2018: Volume 1, Secondary School Students*. Ann Arbor, MI: Institute for Social Research, University of Michigan; 2019.
4. Bélanger RE, Akre C, Kuntsche E, Gmel G, Suris JC. Adding tobacco to cannabis—its frequency and likely implications. *Nicotine Tob Res*. 2011;13(8):746–750.
5. Bélanger RE, Marclay F, Berchtold A, Saugy M, Cornuz J, Suris JC. To what extent does adding tobacco to cannabis expose young users to nicotine? *Nicotine Tob Res*. 2013;15(11):1832–1838.
6. Flatz A, Bélanger RE, Berchtold A, Marclay F, Surìs JC. Assessing tobacco dependence among cannabis users smoking cigarettes. *Nicotine Tob Res*. 2013;15(2):557–561.
7. Baumann MH, Solis E Jr, Watterson LR, Marusich JA, Fantegrossi WE, Wiley JL. Baths salts, spice, and related designer drugs: the science behind the headlines. *J Neurosci*. 2014;34(46):15150–15158.
8. Alam RM, Keating JJ. Adding more "spice" to the pot: a review of the chemistry and pharmacology of newly emerging heterocyclic synthetic cannabinoid receptor agonists. *Drug Test Anal*. 2019;12(3):297–315.
9. Banister SD, Connor M. The chemistry and pharmacology of synthetic Cannabinoid receptor agonists as new psychoactive substances: origins. *Handb Exp Pharmacol*. 2018;252:165–190.
10. Tuesta LM, Fowler CD, Kenny PJ. Recent advances in understanding nicotinic receptor signaling mechanisms that regulate drug self-administration behavior. *Biochem Pharmacol*. 2011;82(8):984–995.
11. Renard J, Krebs MO, Le Pen G, Jay TM. Long-term consequences of adolescent cannabinoid exposure in adult psychopathology. *Front Neurosci*. 2014;8:361.
12. Fowler CD, Lu Q, Johnson PM, Marks MJ, Kenny PJ. Habenular α5 nicotinic receptor subunit signalling controls nicotine intake. *Nature*. 2011;471(7340):597–601.
13. Acquas E, Di Chiara G. Role of dopamine D1 receptors in the control of striatal acetylcholine release by endogenous dopamine. *Neurol Sci*. 2001;22(1):41–42.
14. Solinas M, Scherma M, Tanda G, Wertheim CE, Fratta W, Goldberg SR. Nicotinic facilitation of delta9-tetrahydrocannabinol discrimination involves endogenous anandamide. *J Pharmacol Exp Ther*. 2007;321(3):1127–1134.
15. Polissidis A, Galanopoulos A, Naxakis G, Papahatjis D, Papadopoulou-Daifoti Z, Antoniou K. The cannabinoid CB1 receptor biphasically modulates motor activity and regulates dopamine and glutamate release region dependently. *Int J Neuropsychopharmacol*. 2013;16(2):393–403.
16. Nisell M, Nomikos GG, Hertel P, Panagis G, Svensson TH. Condition-independent sensitization of locomotor stimulation and mesocortical dopamine release following chronic nicotine treatment in the rat. *Synapse*. 1996;22(4):369–381.
17. Parrott AC. Nicotine psychobiology: how chronic-dose prospective studies can illuminate some of the theoretical issues from acute-dose research. *Psychopharmacology (Berl)*. 2006;184(3–4):567–576.
18. Kandel D. Stages in adolescent involvement in drug use. *Science*. 1975;190(4217):912–914.
19. Kota D, Robinson SE, Imad Damaj M. Enhanced nicotine reward in adulthood after exposure to nicotine during early adolescence in mice. *Biochem Pharmacol*. 2009;78(7):873–879.
20. Nesil T, Kanit L, Collins AC, Pogun S. Individual differences in oral nicotine intake in rats. *Neuropharmacology*. 2011;61(1–2):189–201.
21. Fowler CD, Kenny PJ. Intravenous nicotine self-administration and cue-induced reinstatement in mice: effects of nicotine dose, rate of drug infusion and prior instrumental training. *Neuropharmacology*. 2011;61(4):687–698.
22. Matta SG, Balfour DJ, Benowitz NL, et al. Guidelines on nicotine dose selection for *in vivo* research. *Psychopharmacology (Berl)*. 2007;190(3):269–319.
23. Weaver MT, Geier CF, Levin ME, Caggiula AR, Sved AF, Donny EC. Adolescent exposure to nicotine results in reinforcement enhancement but does not affect adult responding in rats. *Drug Alcohol Depend*. 2012;125(3):307–312.

24. Shram MJ, Funk D, Li Z, Lê AD. Nicotine self-administration, extinction responding and reinstatement in adolescent and adult male rats: evidence against a biological vulnerability to nicotine addiction during adolescence. *Neuropsychopharmacology*. 2008;33(4):739–748.
25. Lee TT, Gorzalka BB. Timing is everything: evidence for a role of corticolimbic endocannabinoids in modulating hypothalamic-pituitary-adrenal axis activity across developmental periods. *Neuroscience*. 2012;204:17–30.
26. Yuan M, Cross SJ, Loughlin SE, Leslie FM. Nicotine and the adolescent brain. *J Physiol*. 2015;593(16):3397–3412.
27. Cross SJ, Linker KE, Leslie FM. Sex-dependent effects of nicotine on the developing brain. *J Neurosci Res*. 2017;95(1–2):422–436.
28. Pushkin AN, Eugene AJ, Lallai V, et al. Cannabinoid and nicotine exposure during adolescence induces sex-specific effects on anxiety- and reward-related behaviors during adulthood. *PLoS One*. 2019;14(1):e0211346.
29. Bortolato M, Bini V, Frau R, et al. Juvenile cannabinoid treatment induces frontostriatal gliogenesis in Lewis rats. *Eur Neuropsychopharmacol*. 2014;24(6):974–985.
30. Raver SM, Keller A. Permanent suppression of cortical oscillations in mice after adolescent exposure to cannabinoids: receptor mechanisms. *Neuropharmacology*. 2014;86:161–173.
31. Kirschmann EK, Pollock MW, Nagarajan V, Torregrossa MM. Effects of adolescent Cannabinoid self-administration in rats on addiction-related behaviors and working memory. *Neuropsychopharmacology*. 2016;42(5):989–1000.
32. Fowler CD, Tuesta L, Kenny PJ. Role of alpha5* nicotinic acetylcholine receptors in the effects of acute and chronic nicotine treatment on brain reward function in mice. *Psychopharmacology (Berl)*. 2013;229:503–513.
33. Kutlu MG, Braak DC, Tumolo JM, Gould TJ. Adolescent mice are less sensitive to the effects of acute nicotine on context pre-exposure than adults. *Brain Res*. 2016;1642:445–451.
34. Chadwick B, Miller ML, Hurd YL. Cannabis use during adolescent development: susceptibility to psychiatric illness. *Front Psychiatry*. 2013;4:129.
35. Chen E, Lallai V, Sherafat Y, et al. Altered baseline and nicotine-mediated behavioral and cholinergic profiles in ChAT-Cre mouse lines. *J Neurosci*. 2018;38(9):2177–2188.
36. Pidoplichko VI, DeBiasi M, Williams JT, Dani JA. Nicotine activates and desensitizes midbrain dopamine neurons. *Nature*. 1997;390(6658):401–404.
37. Portugal GS, Wilkinson DS, Turner JR, Blendy JA, Gould TJ. Developmental effects of acute, chronic, and withdrawal from chronic nicotine on fear conditioning. *Neurobiol Learn Mem*. 2012;97(4):482–494.
38. Abreu-Villaça Y, Correa-Santos M, Dutra-Tavares AC, et al. A ten fold reduction of nicotine yield in tobacco smoke does not spare the central cholinergic system in adolescent mice. *Int J Dev Neurosci*. 2016;52:93–103.
39. Bambico FR, Nguyen NT, Katz N, Gobbi G. Chronic exposure to cannabinoids during adolescence but not during adulthood impairs emotional behaviour and monoaminergic neurotransmission. *Neurobiol Dis*. 2010;37(3):641–655.
40. Jobson CLM, Renard J, Szkudlarek H, et al. Adolescent nicotine exposure induces dysregulation of mesocorticolimbic activity states and depressive and anxiety-like prefrontal cortical molecular phenotypes persisting into adulthood. *Cereb Cortex*. 2019;29(7):3140–3153.
41. Bonnie RJ, Stratton K, Kwan LY, eds. *Public Health Implications of Raising the Minimum Age of Legal Access to Tobacco Products*. Washington, DC: National Academies Press; 2015. https://www.ncbi.nlm.nih.gov/books/NBK310407/ Accessed January 1, 2020.
42. Lee TT, Hill MN, Lee FS. Developmental regulation of fear learning and anxiety behavior by endocannabinoids. *Genes Brain Behav*. 2016;15(1):108–124.
43. Castañé A, Valjent E, Ledent C, Parmentier M, Maldonado R, Valverde O. Lack of CB1 cannabinoid receptors modifies nicotine behavioural responses, but not nicotine abstinence. *Neuropharmacology*. 2002;43(5):857–867.
44. Cossu G, Ledent C, Fattore L, et al. Cannabinoid CB1 receptor knockout mice fail to self-administer morphine but not other drugs of abuse. *Behav Brain Res*. 2001;118(1):61–65.
45. Beard E, Shahab L, Cummings DM, Michie S, West R. New pharmacological agents to aid smoking cessation and tobacco harm reduction: what has been investigated, and what is in the pipeline? *CNS Drugs*. 2016;30(10):951–983.
46. Lefever TW, Marusich JA, Antonazzo KR, Wiley JL. Evaluation of WIN 55,212-2 self-administration in rats as a potential cannabinoid abuse liability model. *Pharmacol Biochem Behav*. 2014;118:30–35.
47. Spencer S, Neuhofer D, Chioma VC, et al. A model of Δ9-tetrahydrocannabinol self-administration and reinstatement that alters synaptic plasticity in nucleus accumbens. *Biol Psychiatry*. 2018;84(8):601–610.
48. Freels TG, Baxter-Potter LN, Lugo JM, et al. Vaporized cannabis extracts have reinforcing properties and support conditioned drug-seeking behavior in rats. *J Neurosci*. 2020;40(9):1897–1908.
49. Caspi A, Moffitt TE, Cannon M, et al. Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. *Biol Psychiatry*. 2005;57(10):1117–1127.
50. Dani JA, Harris RA. Nicotine addiction and comorbidity with alcohol abuse and mental illness. *Nat Neurosci*. 2005;8(11):1465–1470.