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

Short Title: Adolescent drug exposure alters adult nicotine intake

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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.

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 seven 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 co-administration. In contrast, adult females demonstrated an opposing effect in which adolescent cannabinoid and nicotine co-exposure resulted in decreased nicotine intake compared to the nicotine only and control groups. Furthermore, after maintaining nicotine self-administration across sessions, pre-treatment 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 co-exposure 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 (e.g., THC and synthetic 'spice' cannabinoids) during development, with further implications for the effectiveness of therapeutic interventions based on prior drug exposure in youth.

KEYWORDS: Adolescent, Cannabis, Mouse, Nicotine, Self-Administration

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 neurocircuitries¹. Thus, this study represents the first to begin deciphering the co-consumption 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 products². Of further concern, 38.6% of these students reported using cannabis². 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 second-hand 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 lifetime³. 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 alone⁶. 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 drug, such as synthetic 'spice' cannabinoid agonists for which the majority belong to the aminoalkylindole class, including WIN55-212,2⁷⁻⁹. 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 brain¹³, and similarly, nicotine administration alters endogenous cannabinoid signaling¹⁴. 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 adulthood¹⁹, 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 (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 paradigms²⁰, potentially due to activation of nAChRs expressed in the tongue and/or post-consummatory 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 postnatal days (PND) 25-42 did not alter later nicotine self-administration behavior during early adulthood²³, but it should be noted that the subjects in this study were individually housed and shipped during PND 20-21²³; 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 PND34 and prior to adult testing²⁴, 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 co-exposure 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 postnatal days 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 wildtype 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 h 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

bodyweight, 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, USA) was dissolved in vehicle containing 1% DMSO, 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 per day)³¹. (-)-Nicotine hydrogen tartrate salt (MP Biomedicals, Santa Ana, CA, USA; 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 postnatal day (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 time frame is considered mid-adolescence in rodents, corresponding to ~13-17 in human years²⁶. 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 described²⁸. 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 sec (FR5TO20 sec) schedule of reinforcement. We have previously shown that these adolescent exposure groups do not differ in operant food learning²⁸. Once stable responding was achieved (>25 pellets per session across 3 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 h 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 h 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 2 retractable levers (1 active, 1 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 (PND70). Thereafter, they were examined for differences in cognitive behavior as reported previously²⁸. For these investigations, to ascertain the dose response function, mice were tested according to the established mouse intravenous self-administration protocol²¹. 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 2 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 min 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 cross-over experiment with the single, acute dose of WIN, mice were chronically pre-treated with the same low WIN dose prior to each session across 7 consecutive sessions, and nicotine intake on the seventh session was used to determine the effects of chronic co-exposure during adulthood for all groups. Since the control groups (adolescent vehicle treatment) for the moderate and low dose WIN cohorts exhibited similar effects with pre-treatment, 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 excluded due to death/cannibalization by cagemates (3 female Control, 1 female NIC, 3 female and 1 male NIC/WIN, 2 female NIC/LdWIN, and 1 male LdWIN), and 6 were excluded due to compromised catheter integrity (2 female Control, 2 female NIC, 1 female NIC/LdWIN, 1 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* hypothesis²⁸. Data were analyzed by a t-test, one-way or two-way ANOVA with Prism 7 software (GraphPad, La Jolla, CA, USA), 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* $F_{(3,25)}=2.13$, $p=0.122$; *Dose* $F_{(3,75)}=38.15$, $p<0.0001$; *Interaction* $F_{(9,75)}=2.29$, $p=0.024$). Specifically, the WIN and nicotine/WIN adolescent exposure groups exhibited a significantly increased number of nicotine infusions compared to the control and nicotine adolescent exposure groups ($p<0.05$ for WIN compared to either control or nicotine; $p<0.01$ for nicotine/WIN compared to 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* $F_{(2,19)}=1.06$, $p=0.368$; *Dose* $F_{(3,57)}=15.51$, $p<0.0001$; *Interaction* $F_{(6,57)}=0.845$, $p=0.541$).

In female mice, statistically significant main and interaction effects were found among the control, nicotine, WIN, and co-exposure nicotine and WIN groups (**Figure 2c**) (*Repeated measures two-way ANOVA, Group* $F_{(3,24)}=5.24$, $p=0.006$; *Dose* $F_{(3,72)}=33.44$, $p<0.0001$; *Interaction* $F_{(9,72)}=2.82$, $p=0.007$). The post-hoc analysis revealed an upward shift in the dose response function for the nicotine exposure group, as compared to both the WIN and co-exposure nicotine

and WIN groups. Specifically, at the 0.03 mg/kg/infusion dose, the adolescent nicotine group exhibited a significantly greater number of nicotine infusions than the adolescent WIN ($p < 0.001$) and nicotine/WIN co-exposure ($p < 0.01$) groups. At the moderate 0.1 mg/kg/infusion dose, the nicotine group also demonstrated a statistically significant increase from the WIN group ($p < 0.01$) and nicotine/WIN co-exposure group ($p < 0.001$), and the nicotine/WIN co-exposure group was also significantly decreased compared to the control group ($p < 0.05$). 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* $F_{(2,18)} = 0.42$, $p = 0.662$; *Dose* $F_{(3,54)} = 29.26$, $p < 0.0001$; *Interaction* $F_{(6,54)} = 0.45$, $p = 0.842$).

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*, $F_{(2,32)} = 8.09$, $p = 0.001$; *Post-hoc*, vehicle vs. acute $p < 0.05$,

vehicle vs. chronic $p < 0.01$) (**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, $F_{(2,14)} = 0.37$, $p = 0.695$; WIN, $F_{(2,16)} = 0.61$, $p = 0.554$; Low dose WIN, $F_{(2,12)} = 5.77$, $p = 0.018$; Nicotine and WIN co-exposure, $F_{(2,8)} = 1.75$, $p = 0.234$; Nicotine and low dose WIN co-exposure, $F_{(2,14)} = 2.67$, $p = 0.104$) (**Figures 3b, 3c, 3d, 3e, 3f, 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*, $F_{(2,26)} = 15.94$, $p < 0.0001$) (**Figure 4a**). Specifically, in post-hoc analyses, the vehicle condition exhibited a higher level of nicotine infusions compared to pretreatment with the cannabinoid after one session (acute, $p < 0.05$) and after seven consecutive sessions (chronic, $p < 0.0001$). Further, chronic administration of the cannabinoid agonist induced significantly reduced nicotine intake to a greater extent than the acute condition ($p < 0.05$). 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, $F_{(2,12)} = 3.09$, $p = 0.083$; WIN, $F_{(2,16)} = 2.16$, $p = 0.148$; low dose WIN, $F_{(2,14)} = 2.11$, $p = 0.158$; Nicotine and WIN co-exposure, $F_{(2,10)} = 2.11$, $p = 0.173$; Nicotine and low dose WIN co-exposure, $F_{(2,10)} = 0.27$, $p = 0.771$) (**Figures 4b, 4c, 4d, 4e, 4f, respectively**).

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 co-administration. 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 to 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, pre-treatment 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 drug³⁶, whereas the aversive signaling of nicotine appears to involve the habenulo-interpeduncular pathway¹². 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 signaling³⁷⁻⁴⁰. 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 females⁴², and cannabinoid 1 receptors (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 paradigm²⁸. The co-exposure condition of both nicotine and the moderate dose of WIN also led to similar behavioral profiles as WIN alone in these measures²⁸, 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 to the control females²⁸. Interestingly, CB1R knockout mice are resistant to nicotine-mediated locomotion and CPP, but do not differ in nicotine self-administration, as compared to wildtype 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 co-exposure 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 consumption⁴⁵. 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 behaviors²⁸. 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 (AAI) class, including WIN 55,212-2⁷⁻⁹. In addition, these findings likely have further implications for cannabis exposure. 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 co-exposure conditions result in different intake amounts of each drug, as compared to 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 CBD 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 some THC e-cigarette vapes on the market do not necessarily contain CBD, 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 co-exposure 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 use⁴⁹, a finding that is of further relevance given the very high comorbidity found between schizophrenia and nicotine dependence⁵⁰. 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.

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Figure Legends

Figure 1. Schematic outline of the experimental design. (a) All mice were treated in adolescence with nicotine (NIC), WIN55,212-2 (WIN), NIC/WIN co-exposure, or vehicle control. WIN was administered at either a low or high dose for the single and co-exposure conditions. After PND70, mice began testing for subsequent examination of intravenous nicotine self-administration across doses. **(b)** After reestablishing baseline responding on the 0.1 mg/kg/infusion nicotine dose, mice were then pre-treated across sessions with vehicle or low dose WIN in a cross-over design. Thereafter, mice were examined with chronic low dose WIN and nicotine self-administration co-exposure for 7 consecutive testing sessions. Finally, after again re-establishing baseline responding, mice were transitioned to respond for saline infusions in the absence of nicotine.

Figure 2. Male and female mice exposed to the cannabinoid agonist during adolescence exhibit opposing effects on nicotine self-administration in adulthood. (a-b) Male intravenous nicotine self-administration dose response function. **(a)** Following exposure to the cannabinoid agonist WIN 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 to the vehicle control and nicotine only (NIC) groups. A similar increase in nicotine intake was also found with nicotine and WIN co-exposure (NIC/WIN) at this dose compared to both the vehicle and NIC groups. (n=6-8/group) *p<0.05 Control vs. WIN, and NIC vs. WIN; **p<0.01 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). Data represent mean values \pm SEM. **(c-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 to the adolescent-exposed cannabinoid agonist WIN groups, either alone or with nicotine co-exposure. The co-exposure 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<0.01 NIC vs. WIN or NIC/WIN; *p<0.05 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).

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 pre-administration and following seven consecutive days of treatment (chronic) in adulthood (n=17) *p<0.05, **p<0.01 compared to 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)** co-exposure of nicotine and the cannabinoid agonist WIN (n=5), or **(f)** co-exposure of nicotine and the low dose of the cannabinoid agonist WIN (n=8).

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 pre-administration and with chronic exposure (n=14). *p<0.05 for vehicle vs acute WIN, and acute WIN vs chronic WIN, ****p<0.0001 for vehicle vs 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)** co-exposure of nicotine and the cannabinoid agonist WIN (n=6), or **(f)** co-exposure of nicotine and the low dose of the cannabinoid agonist WIN (n=6).

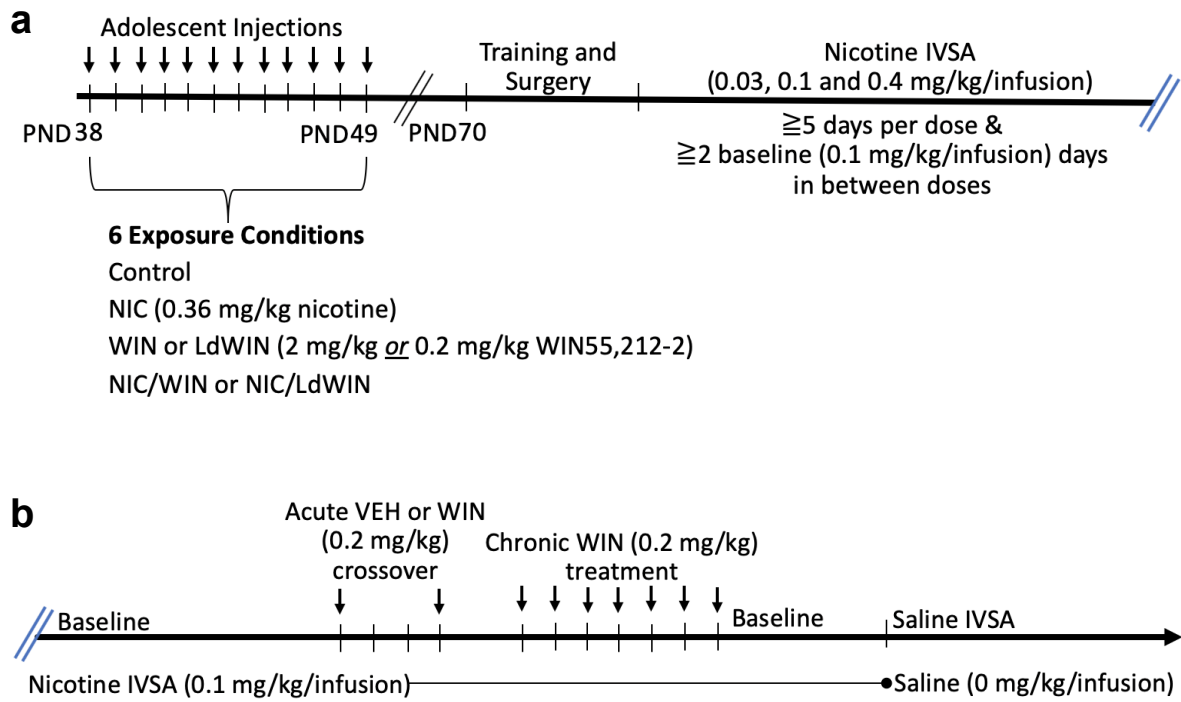
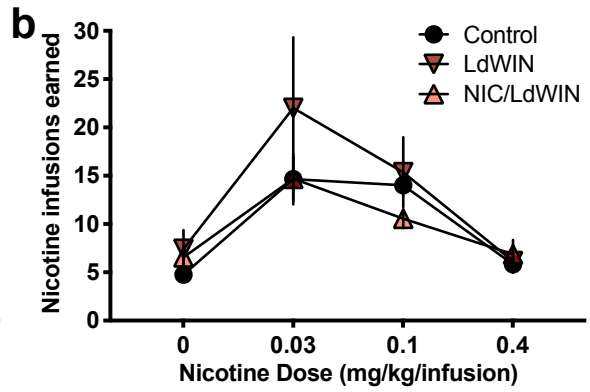
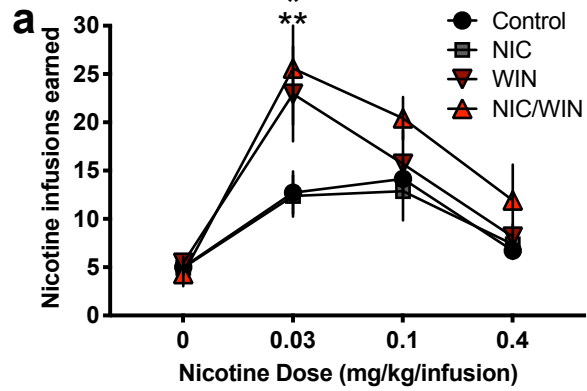


Figure 1

Male



Female

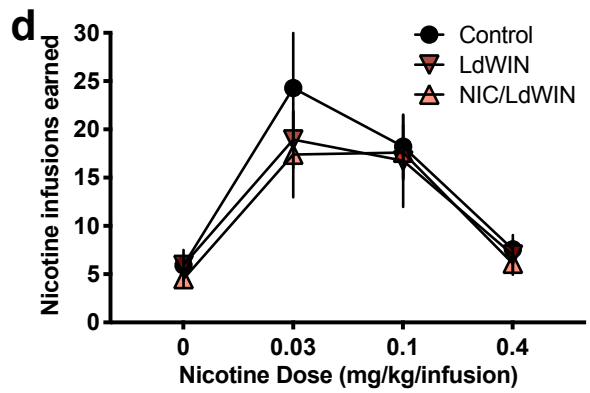
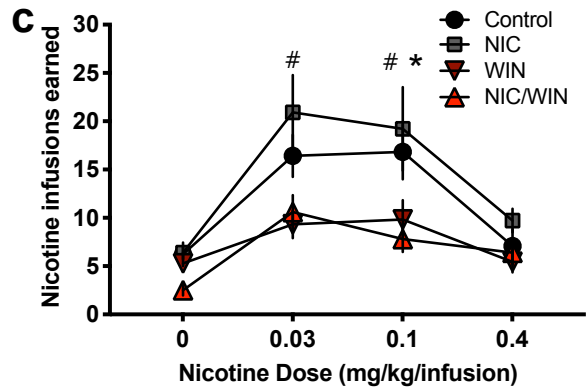
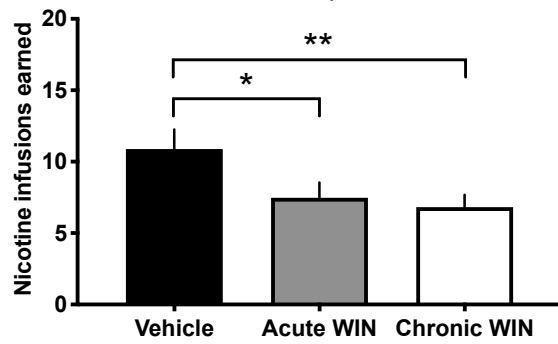


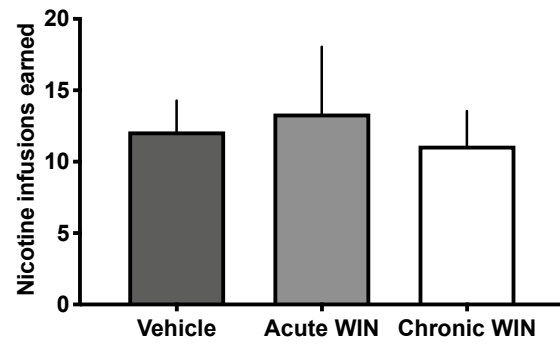
Figure 2

Male

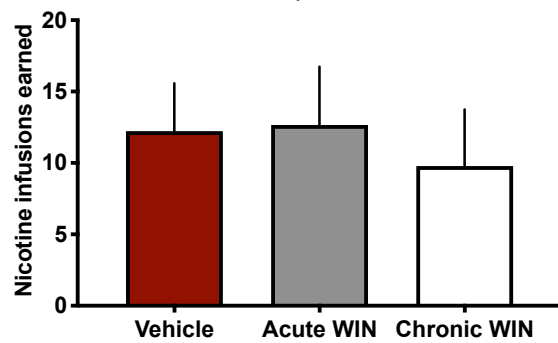
a Control Adolescent Group



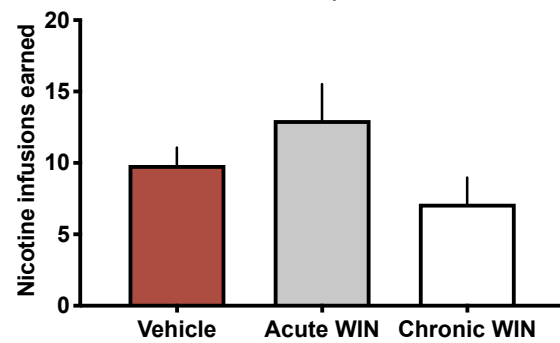
b NIC Adolescent Group



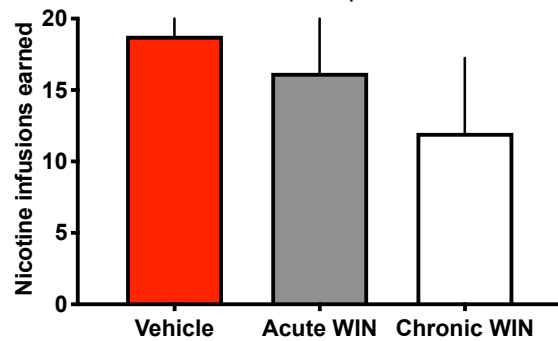
c WIN Adolescent Group



d LdWIN Adolescent Group



e NIC/WIN Adolescent Group



f NIC/LdWIN Adolescent Group

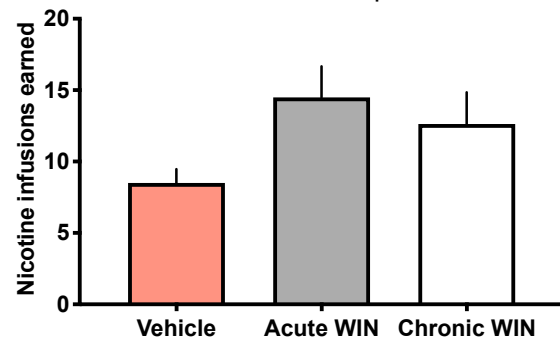
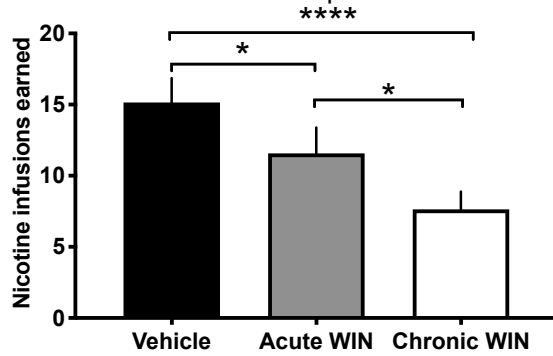


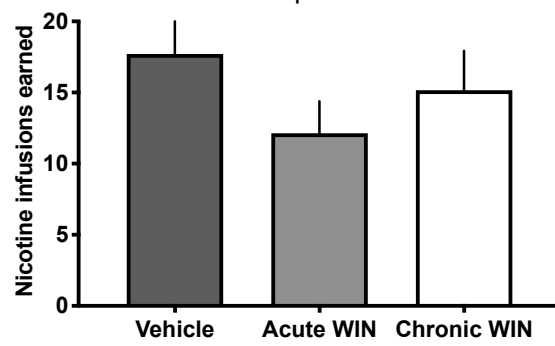
Figure 3

Female

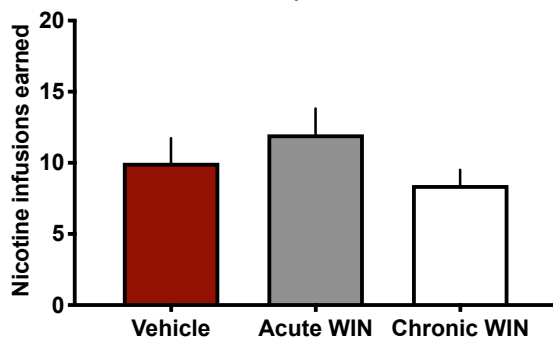
a Control Adolescent Group



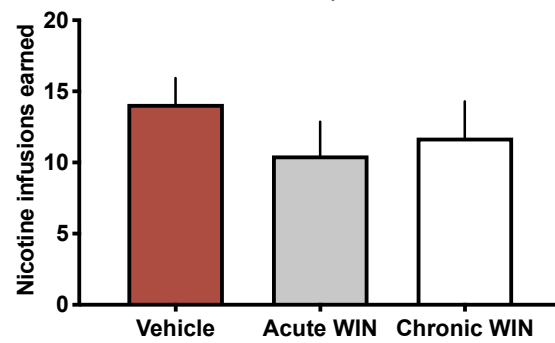
b NIC Adolescent Group



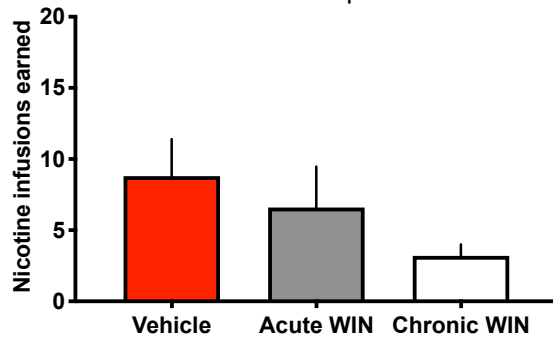
c WIN Adolescent Group



d LdWIN Adolescent Group



e NIC/WIN Adolescent Group



f NIC/LdWIN Adolescent Group

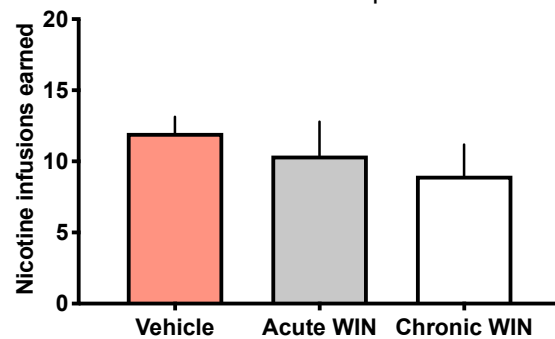


Figure 4