2022-05-01

Sex And Age Differences In Approach Behavior Toward A Port That Delivers Nicotine Vapor

Veronika Evangelina Espinoza
The University of Texas at El Paso

SEX AND AGE DIFFERENCES IN APPROACH BEHAVIOR TOWARD A PORT THAT DELIVERS NICOTINE VAPOR

VERONIKA EVANGELINA ESPINOZA

Master's Program in Experimental Psychology

APPROVED:

Laura E. O'Dell, Ph.D., Chair

Ian A. Mendez, Ph.D.

Sergio D. Iñiguez, Ph.D.

Arshad M. Khan, Ph.D.

Stephen L. Crites, Jr., Ph.D.

Dean of the Graduate School

Dedication

Dedicated to my family: Caroline Mendoza, Alejandro Mendoza, and Gabrel Espinoza, and my boyfriend, Rogelio Dorado.

Thank you for your never-ending love and support.

SEX AND AGE DIFFERENCES IN APPROACH BEHAVIOR TOWARD A PORT THAT DELIVERS NICOTINE VAPOR

by

VERONIKA EVANGELINA ESPINOZA, B.S.

THESIS

Presented to the Faculty of the Graduate School of

The University of Texas at El Paso

in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF ARTS

Department of Psychology

THE UNIVERSITY OF TEXAS AT EL PASO

May 2022

Acknowledgements

First and foremost, I would like to thank my mentor, Dr. Laura O'Dell for her patience, guidance, and never-ending support. She has not only made an impact on my budding career as a scientist but has really made an impact on my personal life as well. I will never forget the first time we met in person and how kind and welcoming you were. You not only opened up your laboratory and team to me, but also your home. Even though I was 700+ miles away from my own, you made me feel like El Paso and UTEP could be my second home. Graduate school has been tough, to say the least, but I am deeply grateful and fortunate to have such a genuine and caring soul on my side, and I could not thank you enough. I would also like to express my gratitude to Dr. Ian Mendez, who has been patient enough to let me use his laboratory equipment to continue pursuing my love for adolescent research as a graduate student. I also would like to thank my other committee members, Dr. Sergio Iñiguez and Dr. Arshad Khan for challenging me in my studies and encouraging me to think outside the box. To my family, thank you for your never-ending love and support. To my mother, who has been my own personal cheerleader since day one, I could never thank you enough for always pushing me to reach for the stars. Also, it has been a privilege to work alongside some of the coolest colleagues, Dr. Luis Carcoba, Dr. Bryan Cruz, Dr. Felix Matos, Dr. Kevin Uribe, Sebastian Ortegon, and Priscilla Giner. My deepest thanks must go to the best undergraduates a graduate student could ever have, Isabella Liano, Alec Rodela, and Paola Correa. Your friendship and hard work have never gone unnoticed and I do not think I could have done it without your help and continued support. Finally, to my loving and supportive boyfriend Rogelio Dorado, I do not think words can explain how much you have made an impact on my life. Your patience, encouragement, and commitment continue to push me to succeed every day. Your never-ending love and support do not go unnoticed.

Abstract

The goal of our laboratory is to study the mechanisms that promote nicotine use, particularly in vulnerable populations such as adolescents and females. Thus, the purpose of this thesis was to characterize age and sex differences in the motivational/rewarding effects of nicotine (Aim 1) and withdrawal behavior (Aim 2). To more closely model human use patterns, the present study employed nicotine vapor methods involving passive exposure for 14 days in adolescent and adult female and male rats. Age and sex differences in approach behavior (nosepokes) were assessed in a port that delivered nicotine plumes on Day 1 and 14. Controls received ambient air. After the final session, rats received a nicotinic receptor antagonist to precipitate withdrawal. Then, physical signs, anxiety-like behavior, and plasma levels of cotinine (a nicotine metabolite) were assessed. Over time, females displayed a larger increase in approach behavior to the nicotine port than males, an effect that was larger in adolescents. Adolescents displayed more total physical signs of withdrawal and grooming behavior than adults, an effect that is likely related to high levels of nosepoke responses in adolescent rats. The analysis of individual signs revealed that there were no age or sex differences in the withdrawal-induced increases in teeth chatters. However, the withdrawal-induced increase in blinking behavior was higher in adult versus adolescent rats, regardless of sex. There were no group differences in anxiety-like behavior. Despite the lack of overall group differences, a correlational analysis in adolescent females revealed that nosepoke responses were positively correlated with the magnitude of anxiety-like behavior, but not physical signs of withdrawal. Adolescents gained more weight than adults regardless of treatment, and the weight gain was larger in male adolescents. Female adolescents also displayed the highest levels of cotinine than all other groups. These findings suggest that nicotine vapor produces greater motivational effects in adolescent females as compared to their adult and male counterparts.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

1.1 Tobacco use as a public health issue

According to the Centers for Disease Control (CDC) and the World Health Organization (WHO), tobacco use remains the leading cause of preventable death and disease in the United States. With approximately 1 billion smokers worldwide, more than 7 million deaths are caused by tobacco use each year (WHO, 2017). By 2030, it is predicted that if the pattern of smoking does not change, more than 8 million people a year will die from diseases related to tobacco use (WHO, 2011). Unfortunately, the rate at which a tobacco smoker develops a disease is quite high. More than 16 million people in the United States are living with a disease caused by smoking, including heart disease, cardiovascular disease, and lung cancer (Department of Health and Human Services, 2014). Also, it was estimated that the annual cost of cigarette smoking in the United States exceeded $225 billion dollars a year in direct medical care for adults (Xu et al., 2015). Although there are 4,800 chemicals in tobacco products, nicotine is the main psychoactive reinforcing compound that motivates smoking behavior (Balfour, 2004). Despite the collection of negative health consequences associated with smoking, a greater understanding is warranted to examine how other factors may contribute to tobacco use that remain problematic. Thus, there is a strong dire need to examine the underlying mechanisms that promote tobacco use in vulnerable populations.

1.2 Nicotine produces rewarding effects and withdrawal symptoms following chronic exposure

Much work has established that tobacco use is largely motivated by the presence of nicotine, which produces dependence in humans and rodent models (Balfour, 2004; Pogocki et al., 2007; Pontieri et al., 1996; U.S. Department of Health and Human Services, 2014). Current theories suggest that tobacco use is motivated by both the positive and negative reinforcement

effects of nicotine (George and Koob, 2017). When nicotine is consumed, it produces acute pleasurable/euphoric effects as well as relaxation, enhanced attentional processes, and motor activation (Benowitz, 1996; Heishman et al., 2010; Hukkanen et al., 2005; Kaye et al., 2014; Le Foll and Goldberg, 2009; Pomerleau & Pomerleau, 1992). Indeed, the recent rise in the use of electronic cigarettes (e-cigarettes) suggests that nicotine produces strong motivational effects (Drope et al., 2017; Yoong et al., 2019). When nicotine is consumed chronically, a withdrawal syndrome emerges during abstinence. The withdrawal syndrome consists of physical symptoms as well as negative affective states that include nausea, headaches, sleep disturbances, irritability, depression, anxiety, and difficult concentrating (Hatsukami et al., 1989; Heishma et al., 2004; Hogle et al., 2006; Hughes et al., 1992; Hughes, 2007; Pauly, 2008; Perkins et al., 2009; Shiffman et al., 2005). The abstinence syndrome plays a major role in continued nicotine use and relapse behavior to avoid negative symptoms that emerge during withdrawal (Baker et al., 2004; Battista et al., 2008; Hughes, 2007, O'Dell & Torres, 2014; Piper et al., 2011). Given the importance of nicotine reward and withdrawal to driving nicotine use, the present work compared age and sex differences in approach behavior (Aim 1) and withdrawal severity (Aim 2) following chronic nicotine exposure.

1.3 E-cigarette use in vulnerable populations

In 2007, e-cigarettes were introduced as a smoking cessation tool that delivers nicotine to alleviate withdrawal symptoms during abstinence. Unfortunately, there has been an epidemic rise in recreational e-cigarette use and an increase in dual use with traditional cigarettes (King et al., 2015; Pepper & Brewer, 2014). There is also growing concern that e-cigarettes provide a "gateway" for greater vulnerability to nicotine use, particularly in adolescents and females. Indeed, adolescents who use e-cigarettes are up to four times more likely to smoke combustible cigarettes

regularly as adults, a relationship that is stronger in females versus males (Chen et al., 2017). With regard to sex differences, women use two-fold higher concentrations of nicotine and display greater symptoms of nicotine dependence than men (Pang et al., 2020). Another report found that women attribute their e-cigarette use to stress reduction and alleviation of withdrawal, whereas men attribute their use to pleasurable effects (Pineiro et al., 2016). Women who smoke are also more likely to have tried e-cigarettes than men (Zhu et al., 2013). This is concerning because women are more susceptible than men to the long-term consequences of smoking, which include reproductive problems, pulmonary disease, and cancer (Kong & Krishnan-Sarin, 2017). Women also face greater challenges than men when attempting to quit smoking. During abstinence, women display greater anxiety, depression, craving, and higher levels of the stress biomarker cortisol than men (Perkins et al., 2000). Existing cessation strategies attempt to reduce withdrawal symptoms via nicotine replacement therapy or administration of partial nicotinic acetylcholine receptor (nAChR) agonists. Unfortunately, these interventions are less effective in women versus men (Bottorff et al., 2012; Cepeda-Benito et al., 2004). Preclinical studies are needed to provide a deeper understanding of underlying mechanisms that promote age and sex differences in nicotine use, particularly with rodent models that more closely model nicotine use patterns in humans.

1.4 Pre-clinical studies that lay the foundation for this thesis

Rodent models have provided an important research tool for evaluating the factors that promote nicotine use (FDA, 2012). Previous work has studied the behavioral and neurochemical effects of nicotine in rodents following intravenous (IV) or oral self-administration, passive subcutaneous (SC) or intraperitoneal (IP) injections, and surgical implantation of an osmotic pump that delivers nicotine continuously (Matta et al., 2007; O'Dell & Khroyan, 2009). Most of the prior work has studied withdrawal in rodents by implanting a subcutaneous pump that delivers

nicotine and then removing the pump or administering a nicotinic acetylcholine receptor antagonist (such as mecamylamine) to precipitate withdrawal. The pump model is limited regarding surgical interventions, the larger size of the pump in adolescent versus adult and female versus male rats, and continuous delivery of nicotine that does not mimic the repeated abstinence periods in human use patterns. Given these limitations, recent efforts have focused on developing nicotine inhalation methods to more closely model emerging nicotine use trends in clinical populations (discussed in Miliano et al., 2020). Emerging work has established that inhalation methods induce nicotine dependence in rats (George et al., 2010; Gilpin et al., 2014; Javadi-Paydar et al., 2019; Kallupi et al., 2019; Montanari et al., 2020) and mice (Lefever et al., 2017; Ponzoni et al., 2015). This work has revealed that chronic nicotine vapor exposure produces dependence in rats, as demonstrated by the emergence of physical signs during withdrawal from 7-14 days of passive exposure to nicotine vapor in rats (George et al., 2010; Gilpin et al., 2014; Javadi-Paydar et al., 2019; Kallupi et al., 2019; Montanari et al., 2020). Prior work using nicotine vapor methods in rats has utilized male adult rodents, leaving remaining questions regarding age and sex differences produced by chronic exposure to nicotine vapor. To address this issue, the present study assessed nosepoke responses in a port that delivered nicotine plumes in a passive vapor inhalation system. Following nicotine vapor exposure, age and sex differences in physical signs and anxiety-like behavior were compared following precipitated withdrawal. Group differences in weight gain and plasma levels of cotinine (a nicotine metabolite) were also assessed on the final day of the vapor regimen.

1.5 Aims

Age and sex differences in the behavioral effects of nicotine reward and withdrawal have not been explored. Thus, this Master's thesis project characterized age and sex differences in the motivational/rewarding effects of nicotine (Aim 1) and withdrawal symptoms (Aim 2) produced

by chronic nicotine vapor exposure in female and male adolescent and adult rats. The motivational effects of nicotine were assessed by measuring approach behavior to a port that delivers nicotine plumes in a passive electronic nicotine vapor inhalation system. Following chronic nicotine exposure, withdrawal-induced group differences were then assessed in physical signs, anxiety-like behavior, and serum levels of cotinine. Withdrawal was studied following administration of mecamylamine, a non-selective nicotinic receptor antagonist. This was done to elicit a discrete time point of withdrawal that has been shown in our laboratory to produce sex differences (O'Dell & Torres, 2014).

1.6 Hypotheses and rationale

For Aim 1, we ***hypothesized*** that adolescent female rats would experience a more heightened rewarding effect of nicotine vapor, as measured as greater approach behavior to the port that delivers nicotine in adolescent females as compared to their adult counterparts. The ***rationale*** for our hypothesis is based on previous evidence showing that female adolescent rats display a greater magnitude of place preference produced by nicotine as compared to adolescent males (Torres et al., 2008; Torres et al., 2009). Self-administration studies focused on age differences in females have also found two-fold higher levels of nicotine intake (Levin et al., 2003) and faster acquisition of nicotine self-administration (Chen et al., 2007) in adolescent female versus adult female rats.

For Aim 2, we ***hypothesized*** that adult rats would display greater physical signs of withdrawal and serum cotinine levels as compared to their adolescent counterparts. The ***rationale*** for our hypothesis is based on previous evidence from our laboratory showing that adult rats display greater physical signs of withdrawal and serum cotinine levels produced by nicotine as compared to adolescent rats (O'Dell, 2009; Torres et al., 2013). Additionally, we ***hypothesized***

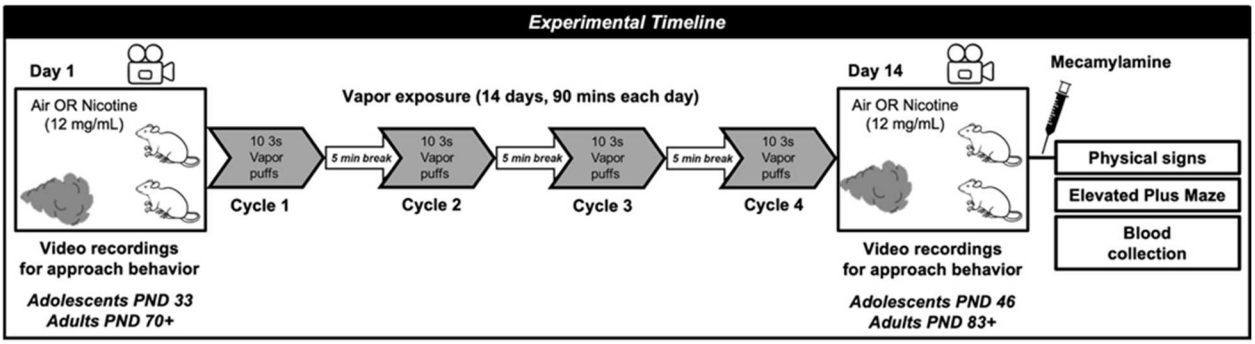
that adult females would experience greater withdrawal-induced anxiety-like behavior as compared to their adult male counterparts. The ***rationale*** for our hypothesis is based on previous work showing that female adult rats display greater anxiety-like behavior than adult males (Torres et al., 2013). Subsequent studies revealed that ovariectomized adult female rats displayed less anxiety-like behavior and corticosterone levels relative to intact females (Flores et al., 2020). Together, this work suggests that the heightened withdrawal severity in adult females is modulated by the presence of ovarian hormones. Thus, we ***hypothesized*** that adolescent females would display less withdrawal severity given that their ovarian hormones are not yet developed.

Chapter 2: Methods

2.1 Subjects

Adult female (n=6 nicotine and n=6 control), adult male (n=6 nicotine and n=6 control), adolescent female (n=6 nicotine and n=6 control), and adolescent male (n=6 nicotine and n=6 control) rats were used. The rats were bred in house from an outbred stock of Wistar rats (Envigo, Inc., Indianapolis, IN). On postnatal day (PND) 21, the rat pups were weaned and pair-housed with a same-sex litter mate for the remainder of the study. The rats were housed in a humidity- and temperature- controlled (22°C) vivarium on a reverse 12-hour light/dark cycle (lights off between 8:00 a.m. and 8:00 p.m.) with access to water and food *ad libitum*. All procedures were approved by the UTEP Institutional Animal Care and Use Committee in compliance with the Guide for the Care and Use of Animals (National Research Council, 2010).

2.2 Experimental procedures



The inset depicts our vapor exposure regimen and test procedures. Before the start of the study, the rats were handled for at least 5 minutes each day in the vivarium for 5 days. The exposure procedures utilized a Benchtop Passive E-Vape Inhalation system from La Jolla Alcohol Research Inc. (La Jolla, CA). Separate pairs of rats were exposed to either nicotine (12 mg/mL) or ambient air (controls) for 90 minutes each day for 14 consecutive days. The pairs of rats were derived from the same group condition (i.e., a female adolescent with another female adolescent) and they

remained with the same partner throughout the exposure procedure. Our decision to use ambient air for the control condition was based on previous studies from our laboratory showing that PG/VG elicits behavioral effects, such as changes in riskiness, that could impact our assessments of approach behavior (Giner et al., 2022). Other laboratories have also used ambient air as a control condition in nicotine vapor studies in rats (Gilpin et al., 2014).

Each day, the rats were exposed to 90-minute sessions consisting of four cycles, with 5-minute inter-cycle intervals. For each cycle, nicotine e-liquid was heated to 400°F for a 3-second puff delivery, occurring every 2 minutes and 10 times per cycle, for a total of 40 puff deliveries per day. Each cycle duration was 18 minutes and 30 seconds. We used flavorless e-liquids containing nicotine in its freebase form in 50/50 vegetable glycerin/propylene glycol (PG/VG) vehicle.

The rationale for our procedures was based largely on prior studies in our laboratory and others using nicotine vapor in rats (Flores et al., 2022; George et al., 2010; Gilpin et al., 2014; Javadi-Paydar et al., 2019; Kallupi et al., 2019; Montanari et al., 2019; Smith et al., 2020). Our e-liquid concentration of nicotine (12 mg/mL) is a moderate concentration that falls within a range of e-liquid concentrations preferred by human e-cigarette users (Etter, 2016; Flouris et al., 2013). Our flavorless e-liquids are purchased in bulk from a commercial vendor (Vapor Vapes Inc, Sand City, CA) that is a popular choice among e-cigarette users. While these liquids provide a better model of human e-cigarette use, it is acknowledged that they may vary in dose and contain contaminants.

The 14-day exposure procedure was done in adolescent rats between PND 33-46 and adult rats between PND 70-88. Two cage mates were exposed in the same chamber throughout the exposure procedure. The exposure system consisted of four sealed chambers (interior dimension

of 14.5" L x 10.5" W x 9.0" H), each with two valve ports. One valve port was connected to a small vacuum that controlled the airflow in the chamber at 0.6 L per minute. The vacuum outlet was connected to a Whatman HEPA filter (Millipore Sigma, Darmstadt, Germany) and onto a house exhaust that safely removed the nicotine from the chambers and outside the testing room. The other valve port was connected via PVC tubing to a modified 4.9-volt TFV4 minitank (Smok Inc, Shenzhen, China) where the nicotine was heated. The minitanks were also linked to a control box that allowed for controlled heating of nicotine e-cigarette liquid (e-liquid). To minimize contamination, the chambers were carefully cleaned after every exposure session, and separate PVC tubing and minitanks were used for air controls and nicotine vapor groups.

2.3 Behavioral testing

During the exposure regimen, nosepokes in the vapor plume delivery port were recorded on Day 1 and 14. The frequency of port contacts (nosepokes) served as an index of approach behavior that was directed at the port where the nicotine plumes were delivered into the chamber. Nosepoke responses were manually scored by an observer that was blind to the rats' treatment condition. The videos were scored two separate times manually to capture nosepoke responses for each individual rat of the pairings. In our figures, individual data points are color matched to allow for comparisons in each pair of rats. We did not observe any interference of nosepoking behavior when assessing rat pairs. More animals are needed to confirm this assertion given that there were only three pairs of rats per group in the present dataset.

At the end of the final vapor exposure session on Day 14, the rats were placed into a clear Plexiglas® cage and moved to a dedicated test room that was well lit. Following a 10-minute acclimation period, the rats received a subcutaneous injection of mecamylamine (3.0 mg/kg) to precipitate nicotine withdrawal. This dose of mecamylamine elicits physical signs of withdrawal

in nicotine-dependent female and male Wistar rats (Torres et al., 2015). Ten minutes later, the physical signs of withdrawal were assessed for an additional 10 minutes. The observed signs included blinks, writhes, body shakes, teeth chatters, gasps, grooming, licks, and ptosis. Each sign was operationally defined in **Table 1** with a description of the regulatory systems believed to modulate each behavior. Multiple successive counts of any sign required a distinct pause between episodes. Ptosis was counted once per minute.

Following our assessments of physical signs, anxiety-like behavior was assessed using elevated plus maze (EPM) procedures. The rats were transported to another dimly lit room and acclimated for 5 minutes. The EPM apparatus consisted of 4 arms (2 closed and 2 open) elevated to a height of 50 cm above the ground. The apparatus was illuminated by a red light suspended from the ceiling. At the beginning of the test, the rats were placed in the center of the EPM facing the open arm. Time spent in the center area and the open versus closed arms was record for 5 minutes. Anxiety-like behavior was operationally defined as an increase in time spent in the closed arms relative to controls. All behavioral measures were assessed by an observer that was unaware of the rats' treatment condition.

2.4 Cotinine levels assessment

Immediately after behavioral testing, the rats were sacrificed, and blood was collected. Nicotine metabolism was assessed indirectly by comparing cotinine (a nicotine metabolite) levels across experimental conditions. The blood was centrifuged for 15 minutes at 5000 x g at 4°C. Serum was extracted and stored in 100 μL aliquots at -80°C. The serum cotinine levels were analyzed using commercially available 96-well plate ELISA kits (OraSure Technologies, Inc., Bethlehem, PA). Standard curves were used to estimate plasma cotinine levels using a Spectra Maxplus spectrophotometer (Molecular Devices Inc, Sunnyvale, CA).

2.5 Statistics

The dependent variables included, nosepoke responses, weight gain, cotinine levels, time spent in the closed arms of the EPM, and physical signs of withdrawal. The nosepoke data and changes in weight were expressed as a percent change from Day 1 to assess time-dependent effects across treatment groups. Multivariate analysis of variance (ANOVA) was used with sex (female versus male), age (adolescent versus adult), and treatment (control versus nicotine) as between-subject factors. For the nosepoke data, time was included in the ANOVA as a within subject factor (Day 1 versus 14). Where appropriate, significant interaction effects were further analyzed using post-hoc comparisons (Fisher's LSD test, $p \leq 0.05$). A Bonferroni correction factor was employed to reduce error inflation with multiple comparisons. The relationship between approach behavior and cotinine levels was assessed using a Pearson correlation coefficient analysis. Data were analyzed using IBM SPSS Statistics for Windows, version 27 (IBM Corp., Armonk, NY). **Table 2** depicts all the statistical analyses. All significant interaction effects were depicted, and only main effects were shown where interaction effects were not discovered.

Chapter 3: Results

3.1 Nosepoke responses

Figure 1 displays nosepokes in control (white bars) and nicotine vapor (grey bars) groups on Day 1 and 14 of the exposure regimen. Overall, the data show that female rats displayed an increase in approach behavior toward a port that delivered nicotine, and this effect was larger in adolescents. The analysis of Day 1 revealed that all rats that received nicotine vapor displayed fewer nosepoke responses than controls (main effect of treatment: $F_{(1,40)}$=13.08; *p=0.001). Also, adolescent rats displayed more nosepoke responses than adults (main effect of age: $F_{(1,40)}$=8.74; $^{@}p$=0.005). The analysis of Day 14 revealed significant interactions between sex and treatment ($F_{(1,40)}$=12.61; p=0.001), sex and age ($F_{(1,40)}$=17.80; p=0.001), and treatment and age ($F_{(1,40)}$=5.61; p=0.02). Post-hoc analyses of these interaction effects revealed that female adolescent controls displayed more nosepoke responses than adult female controls ($^{@}p<0.05$) and adolescent male controls ($^{\dagger}p\leq0.05$). Female adolescent rats that were exposed to nicotine displayed more nosepoke responses than adolescent female controls (*$p\leq0.05$) and their adult female ($^{@}p<0.05$) and adolescent male ($^{\dagger}p\leq0.05$) counterparts. Female adult controls displayed more nosepoke responses than adult male controls ($^{\dagger}p\leq0.05$). Female adults that were exposed to nicotine displayed more nosepoke responses than adult female controls (*$p\leq0.05$) and their adult male counterparts ($^{\dagger}p\leq0.05$). Male adolescent controls displayed more nosepoke responses than adult male controls ($^{@}p<0.05$). Male adolescents that were exposed to nicotine displayed more nosepoke responses than adolescent male controls (*$p\leq0.05$) and their adult male counterparts ($^{@}p<0.05$). Male adult rats that were exposed to nicotine displayed more nosepoke responses than adult male controls (*$p\leq0.05$).

Figure 2 displays nosepoke responses in control (white bars) and nicotine vapor (grey bars) groups on Day 14 expressed as % change from Day 1. Overall, the data show that across time, female rats display a larger increase in approach behavior, and this effect was larger in adolescents. The analysis of adolescents revealed a significant interaction between sex, treatment, and time ($F_{(1,40)}$=8.68; p=.005). The post-hoc analyses revealed that adolescent females exposed to nicotine vapor displayed a greater increase in nosepokes over time than female controls (*$p \leq 0.05$). Also, female adolescents that were exposed to nicotine displayed more nosepoke responses on Day 14 as compared to Day 1 ($^{\#}p \leq 0.05$). On Day 14, female adolescent rats that were exposed to nicotine also displayed more nosepoke responses than adolescent female controls (*$p \leq 0.05$) and their adolescent male counterparts ($^{\dagger}p \leq 0.05$). Female adults that were exposed to nicotine displayed more nosepoke responses on Day 14 as compared to Day 1 ($^{\#}p \leq 0.05$). On Day 14, female adults that were exposed to nicotine also displayed more nosepoke responses than adult female controls (*$p \leq 0.05$) and their adult male counterparts ($^{\dagger}p \leq 0.05$). Male adults that were exposed to nicotine displayed more nosepoke responses than adult male controls (*$p \leq 0.05$).

3.2 Physical signs

Figure 3 displays physical signs of withdrawal in control (white bars) and nicotine vapor (grey bars) groups on Day 14 of the exposure regiment. Overall, the data shows that adolescent rats display more physical signs of withdrawal than adults. An analysis of the sum total of all physical signs revealed a significant interaction between treatment and age ($F_{(1,40)}$=9.24; p=0.004). Post hoc analyses revealed that adolescents exposed to nicotine vapor displayed more physical signs than adults (@p<0.05). Separate ANOVAs were also conducted for individual physical signs that are the most objective behavioral measures of withdrawal. An analysis of teeth chatters revealed a significant main effect of treatment ($F_{(1,40)}$=13.08; p=0.001), with all rats that received

nicotine vapor displaying an increase in teeth chatters relative to controls. A separate analysis of blinks revealed a significant main effect of treatment ($F_{(1,40)}$=103.87; p=0.001), with all rats that were exposed to nicotine vapor displaying an increase in blinks relative to controls. There was also a main effect of age ($F_{(1,40)}$=9.64; p=0.01), with adolescents displaying less blinks than adults regardless of sex or treatment condition. A separate analysis of grooming revealed a significant interaction between treatment and age ($F_{(1,40)}$=24.68; p=0.001). Post hoc analyses revealed that all rats that were exposed to nicotine vapor displayed more grooming behavior as compared to controls (* p<0.05). Also, the adolescent rats that were exposed to nicotine vapor displayed more grooming behavior than adults (@p<0.05).

3.3 Anxiety-like behavior

Figure 4 displays anxiety-like behavior in control (white bars) and nicotine vapor (grey bars) groups on Day 14 expressed as % time spent in the closed arms of the EPM. Overall, the data shows that adolescent and adult rats did not display anxiety-like behavior. An analysis of % time spent in the closed arms revealed no interaction between sex, treatment, and age ($F_{(1,40)}$=0.05; p=0.819). Also, an analysis of % time spent in the closed arms revealed no interaction between treatment and sex ($F_{(1,40)}$=0.001; p=0.98), treatment and age ($F_{(1,40)}$=1.06; p=0.31), and sex and age ($F_{(1,40)}$=0.25; p=0.62). Lastly, an analysis revealed no main effect of treatment ($F_{(1,40)}$=3.20; p=0.08), sex ($F_{(1,40)}$=3.07; p=0.09), or age ($F_{(1,40)}$=0.14; p=0.71). We note that there was a strong trend for treatment and sex, and this analysis would likely have reached statistical significance with more animals in each group.

Although there were no overall group differences in anxiety-like behavior, we wanted to examine the relationship between withdrawal severity and the magnitude of anxiety-like behavior. **Figure 5** displays our correlational analyses between approach behavior and withdrawal-induced

changes in physical signs (top panel) and anxiety-like behavior (bottom panel) in control (open circles with dotted lines) and nicotine vapor (closed circles with solid lines) groups on Day 14. Overall, the results revealed that approach behavior was positively correlated with withdrawal-induced increases in anxiety-like behavior. Specifically, in adolescent females that were exposed to nicotine vapor, nosepoke responses were positively correlated with % time spent in closed arms (r=0.80, *p=0.05). The nosepoke responses in the nicotine-treated group were not correlated with physical signs of withdrawal.

3.4 Body weight

Figure 6 displays changes in body weight in control (white bars) and nicotine vapor (grey bars) groups on Day 14 expressed as % change from Day 1. Overall, the data show that adolescents gain more weight than adults, and the magnitude of weight gain was greater in males. The analysis of adolescent rats revealed a significant interaction between sex and time ($F_{(1,40)}$=6.63; p=0.001). Post-hoc analyses revealed that all adolescent rats gained weight across time ($^{\#}p\leq0.05$), an effect that was larger in males ($^{\dagger}p\leq0.05$). The analysis of adult rats revealed a main effect of time ($F_{(1,40)}$=5.77; p=0.02), with all adult rats gaining some weight across time ($^{\#}p\leq0.05$). Lastly, there was a larger increase in weight gain in adolescent versus adult rats regardless of their treatment condition ($\underline{^{@}p<0.05}$).

3.5 Cotinine levels

Figure 7 displays cotinine levels in control (white bars) and nicotine vapor (grey bars) groups on Day 14. Overall, the data reveal that adolescent females that were exposed to nicotine vapor displayed the largest increase in cotinine levels at the end of the final exposure session. The overall analysis revealed a significant interaction between sex, age, and treatment ($F_{(1,40)}$=8.94; p=0.005). All rats that were exposed to nicotine vapor displayed an increase in cotinine levels

relative to their respective control group (*$p \leq 0.05$). Adolescent females that were exposed to nicotine vapor displayed higher cotinine levels than adult females ($^{@}p < 0.05$) and adolescent males ($^{\dagger}p \leq 0.05$).

Chapter 4: Discussion

4.1 Summary

In summary, our major finding was that female rats displayed greater approach behavior to a port that delivered nicotine vapor as compared to males, and this effect was larger in adolescents. Following precipitated nicotine withdrawal, both female and male adolescent rats displayed a larger increase in all physical signs combined relative to adults. An analysis of the individual signs revealed that withdrawal-induced increases in teeth chatters were similar across age and sex. Blinking behavior was higher in adults versus adolescent rats regardless of sex. There was no significant difference in the magnitude of anxiety-like behavior across all treatment groups during withdrawal. However, a correlational analysis revealed that in adolescent females, approach behavior was positively correlated with the magnitude of anxiety-like behavior, but not physical signs of withdrawal. Over time, adolescent rats gained more weight than adults, and this effect was larger in adolescent males regardless of their treatment condition. Not surprisingly, female adolescents that exhibited the largest amount of nosepoke responses in the port that delivered nicotine also displayed the highest levels of the nicotine metabolite, cotinine.

4.2 Nosepoke responses

A major finding of the present report was that across time adolescent female rats displayed the largest increase in approach behavior toward the delivery site of the nicotine vapor plumes. The time-dependent increase in the magnitude of nosepoke responses is believed to reflect greater nicotine reward-seeking behavior in adolescent females. This interpretation of our nosepoke data is consistent with previous work comparing age and sex differences in the rewarding effects of nicotine. For example, the magnitude of place preference produced by nicotine is larger in female adolescent rats as compared to their male counterparts (Torres et al., 2008; Torres et al., 2009).

Another report using intravenous self-administration procedures revealed that female adolescent rats display two-fold higher levels of nicotine intake relative to female adults (Levin et al., 2007). Adolescent female rats also acquire nicotine self-administration more rapidly and maintain higher levels of nicotine intake than adults (Chen et al., 2007). In a meta-analysis that included all of the existing nicotine self-administration studies in rats, the major conclusion was that the magnitude of nicotine intake is larger in female versus male rats, and the effect size was larger in adolescents (Flores et al., 2019). Thus, the present study supports prior work demonstrating that the reinforcing effects of nicotine are greater in adolescent rats, particularly female adolescents.

The present study extends prior work by showing that the motivational effects of nicotine vapor are stronger in females during the adolescent period of development. Prior reports have compared nicotine vapor self-administration in rodents. One report in adult male mice found that reliable nicotine vapor self-administration required the addition of menthol or flavorants (Cooper et al., 2021). Another report showed stable nicotine vapor self-administration in adult female and male rats, albeit with low discrimination between the active and inactive lever (Smith et al., 2020). Indeed, another report comparing sex differences found that the discriminative stimulus effects of nicotine vapor were lower in females versus males (Lefever et al., 2019). A recent report also revealed that adolescent male rats displayed larger shifts in preference behavior than adult males using a shorter puff duration than was used in the present study (Frie et al., 2020). Another study found that adolescent female mice escalated their consumption of a flavored nicotine solution as compared to male adolescent male mice (Patten et al., 2021). Together with existing work, the present findings suggest that the discriminative stimulus and reinforcing effects of nicotine are enhanced in adolescent female rats. It is noted that adolescent female controls displayed higher nosepoke responses for the port that delivered ambient air. In prior work with self-administration

procedures, we have noted that adolescent rats display higher responding on an inactive lever as compared to adults (Natividad et al., 2013). Thus, in the present study the nosepoke responses in the air port are believed to reflect hyperactivity in young animals. Importantly, our effects appear to be specific to nicotine given that adolescents displayed higher nosepoke responses for the port that delivered nicotine as compared to air controls, suggesting that our effects were motivated by the rewarding effects of nicotine.

4.3 Physical signs of withdrawal

The present study also revealed that there were no sex differences in the physical signs of withdrawal, consistent with previous work in our laboratory using nicotine pump exposure (Correa et al., 2019; Flores et al., 2020; Torres et al., 2013). Our findings are also consistent with another laboratory using similar experimental conditions (Hamilton et al., 2009). The latter report also found that male rats display greater physical signs of withdrawal than females in a dimly lit test room, suggesting that sex differences in physical signs may emerge under certain lighting conditions. A comparison across age groups revealed that adolescents displayed more total physical signs of withdrawal than adults, an effect that is likely related to high levels of nosepoke responses in adolescent rats. Indeed, prior work revealed that the magnitude of precipitated withdrawal signs was positively correlated with the amount of nicotine that was intravenously self-administered (O'Dell et al., 2006) or passively delivered via vapor exposure (George et al., 2010).

Our assessment of physical signs also included an analysis of individual behaviors. The individual signs are shown in **Table 1** with the definition of each behavior and the putative system that modulates these behaviors. The analysis of individual signs revealed that there were no age or sex differences in the withdrawal-induced increases in teeth chatters. The pattern of age and sex differences in grooming behavior was similar to our findings with our analysis of the sum total

physical signs. However, the withdrawal-induced increase in blinking behavior was higher in adult versus adolescent rats, regardless of sex. These results suggest that age and sex differences in the individual behavioral signs of withdrawal can vary from conclusions that are based on total physical signs of withdrawal. The possibility exists that the individual signs reflect a different manifestation of the withdrawal syndrome. In fact, a recent report utilized fiber photometry in the interpeduncular nucleus (IPN) to provide a rapid and time-locked assessment of the behavioral effects of nicotine withdrawal with neural activity in this region (Avelar and George, 2022). Their goal was to compare neural activity in the IPN and collected various behaviors associated with the expression of negative emotional states or coping behaviors, such as grooming. They observed that the IPN GABA neuron activity ramped up before a grooming/scratching episode and rebounded after the grooming episode. They interpreted their data to suggest that these withdrawal-related behaviors emerge as a coping mechanism to reduce the increase in IPN GABA neurons activity that is caused by an increase in anxiety-like behavior during withdrawal. Another report assessed the effects of mecamylamine on neural activity in IPN GABAergic neurons with various behavioral measures in nicotine-dependent mice (Klenowski et al., 2021). They found that somatic symptoms including grooming and scratching reduced IPN GABAergic activity during withdrawal. In the elevated plus maze, used to measure anxiety-like behavior, they found that IPN GABAergic neuron activity was increased in the IPN during open- versus closed-arm exploration during withdrawal. Taken together, the existing literature suggests that GABAergic transmission in the IPN, which is enriched in nicotinic receptors, controls the emotional, physical, and motivational aspects of nicotine withdrawal. Future work is needed to better understand how each individual behavior varies across age and sex during withdrawal. Moreover, future work is needed

to determine whether the different physical signs of withdrawal are modulated via distinct brain mechanisms.

4.4 Anxiety-like behavior

The present study revealed that there were no age and sex differences in anxiety-like behavior. These findings were not consistent with previous work in our laboratory showing that adult females and adolescent males spent more time on the closed arms of the elevated plus maze during nicotine withdrawal as compared to males (Torres et al., 2013). One possible explanation for this discrepancy with our prior work is that the saline controls in the present study displayed more time in the closed arms, suggesting that the present cohort of animals were more stressed than animals in the Torres et al paper. A major goal of this report was to provide insight into the role of nicotine dependence in motivating approach behavior in female and male rats from different age groups. To address this issue, we conducted a correlational analysis between nosepoke responses and the magnitude of withdrawal severity (physical signs of withdrawal and anxiety-like behavior). Our analysis revealed that in female adolescent rats, the magnitude of approach behavior following nicotine vapor exposure was closely associated with the expression of anxiety-like behavior during withdrawal. Interestingly, there was no correlation between approach behavior and physical signs of withdrawal. These findings suggest that adolescent females may be more motivated to seek nicotine following repeated exposure to alleviate negative affective states produced by withdrawal. Prior work in our laboratory has found that withdrawal severity is lower in adolescent versus adult rats that received nicotine via osmotic minipumps (see O'Dell, 2009). Thus, the possibility exists that age differences in withdrawal may vary in procedures involving passive and continuous delivery of nicotine as compared to procedures involving volitional intake of inhaled nicotine vapor.

4.5 Body weight

It is recognized that group differences in body weight have the potential to influence the amount of nicotine that is absorbed. One might expect that following exposure to the same amount of nicotine, a large animal might display lower nicotine levels as compared to a smaller rat due to differences in size. However, in the present study the female adolescent rats displayed higher nosepoke responses and the highest cotinine levels relative to all other groups. The analysis of changes in body weight revealed that adolescent rats gained more weight than adults regardless of treatment. This pattern of results is consistent with a prior self-administration study showing that both female and male adolescent rats gained more weight than adults regardless of their self-administration history (Schassburger et al., 2016). Thus, group differences in body weight do not likely explain the pattern of results in nosepoke responses that were age- and sex-dependent.

4.6 Cotinine levels

The present study found that female adolescents that displayed the largest increase in nosepoke responses in the port that delivered nicotine displayed the highest cotinine levels. Since cotinine is a direct metabolite of nicotine with a longer half-life, the assessments of cotinine served as a biomarker for detecting different levels of nicotine intake across treatment groups. The increased cotinine levels observed in rats that spent more time near the vapor input port is likely driven by higher nicotine intake through inhalation, and in part, through oral and transdermal absorption.

4.7 Limitations

The present study used 90-minute sessions for vapor exposure, which reflects a limited exposure period as compared to e-cigarette use in humans, who inhale e-cigarette vapor throughout the day (Dawkins et al., 2013). Thus, we recognize the need to examine age and

sex differences using longer sessions across an extended period. This is important given prior work showing that the escalation of nicotine intake is observed in rats that are given extended access to nicotine intake with intravenous self-administration procedures (see O'Dell et al., 2007). Indeed, ongoing efforts to establish nicotine vapor self-administration will be critical for studying age and sex differences under voluntarily access to nicotine vapor conditions. Another consideration is that prior work using osmotic pumps revealed that nicotine metabolism is faster in adolescent rats (Torres et al., 2013; Trauth et al., 2000). However, the present study revealed that adolescent females that displayed the highest amount of nosepoke responses also had the highest cotinine levels. We believe that the higher cotinine levels are a direct result of greater nicotine exposure in female adolescent rats. However, future work is needed to better understand age and sex differences produced by nicotine following different routes of administration. Indeed, a prior study found that male rats displayed similar cotinine levels following exposure to nicotine via inhalation or intravenous administration, however, females displayed differences in nicotine metabolism across these different routes of administration (Lallai et al., 2021). Taken together, these findings reveal important sex differences in nicotine metabolism based on the route of exposure, an issue that we will need to carefully consider when we make group comparisons in our nicotine vapor self-administration studies in rats.

Another limitation of this report is that our video recordings were unable to capture facial expressions or ultrasonic vocalizations, both of which can provide opportunities for a micro-structural analysis of affective states. For example, prior work has shown that rodents display facial responses that signal disgust or liking versus wanting processes (Khan et al., 2020). Also, prior work has revealed that rats emit ultrasonic vocalizations in the 22-25 kHz range during withdrawal from opiates and cocaine (Covington and Miczek, 2003). Thus, more detailed video

recordings and/or ultrasonic vocalizations might be incorporated in future work aimed at understanding the motivation for nosepoke responses for nicotine vapor in rats.

4.8 Conclusion

The present work lays a foundation for ongoing efforts to establish nicotine vapor self-administration, led by the Mendez laboratory. Our plan for future studies is to utilize a two-phase procedure whereby the rats will first receive passive delivery of nicotine vapor before the animals are allowed to nosepoke for additional nicotine plumes. Based on the present findings, it is expected that the rats will readily self-administer nicotine vapor given that over time they display an increase in nosepokes in the port that delivered the nicotine plumes. It is also expected that females will display the quickest acquisition of instrumental responding and the highest level of self-administration behavior, particularly if the second phase of the study is initiated during adolescence. Our predictions are supported by prior work showing that female rats display greater approach responses to nicotine-predictive cues than males (Stringfield et al., 2019). The present work is an important first step in our long-term goal of elucidating the mechanisms that modulate age and sex differences in nicotine use.

In conclusion, there are clinical implications of the present work. First, our work suggests that nicotine produces strong motivational/rewarding effects that largely motivate e-cigarette use during adolescence. Also, it is likely that nicotine use may enhance the positive effects of other substances commonly abused in adolescents, including alcohol (Schmid et al., 2007). Additionally, the pleasurable effects of nicotine may facilitate risky behavior and social interaction that elicits strong positive affective states in adolescents. This implies that an important strategy for reducing nicotine use in adolescence should focus on avoidance strategies to reduce experimentation and access to e-cigarettes in young persons. Regarding withdrawal, the present study revealed age

differences in nicotine withdrawal severity. The current diagnostic criteria for nicotine dependence are based largely on adults. Thus, these criteria may need to be reconsidered for adolescent nicotine users. Also, the current cessation treatments that focus on alleviating withdrawal may produce different clinical outcomes in nicotine users from different demographic age groups.

References

Avelar, A. J., & George, O. (2022). How nicotine withdrawal symptoms fight each other: interpeduncular GABA neuron activity dynamically controls negative affect vs. coping behavior. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 47(3), 617–618.

Balfour D. J. (2004). The neurobiology of tobacco dependence: a preclinical perspective on the role of the dopamine projections to the nucleus accumbens [corrected]. Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco, 6(6), 899–912.

Baker, T. B., Brandon, T. H., Chassin, L., (2004). Motivational influences on cigarette smoking. Annu. Rev. Psychol., 55, 463–491.

Battista, S. R., Stewart, S. H., Fulton, H. G., Steeves, D., Darredeau, C., Gavric, D., (2008). A further investigation of the relations of anxiety sensitivity to smoking motives. Addictive Behaviors, 33(11), 1402–1408.

Bedard, P., & Pycock, C. J. (1977). "Wet-dog" shake behaviour in the rat: a possible quantitative model of central 5-hydroxytryptamine activity. Neuropharmacology, 16(10), 663–670.

Benowitz, N. L., (1996). Pharmacology of nicotine: addiction and therapeutics. Annual Review of Pharmacology and Toxicology, 36(1), 597–613.

Bottorff, J. L., Haines-Saah, R., Oliffe, J. L., & Sarbit, G. (2012). Gender influences in tobacco use and cessation interventions. The Nursing clinics of North America, 47(1), 55–70.

Cepeda-Benito, A., Reynoso, J. T., & Erath, S. (2004). Meta-analysis of the efficacy of nicotine replacement therapy for smoking cessation: differences between men and women. Journal of consulting and clinical psychology, 72(4), 712–722.

Chen, H., Matta, S. G., & Sharp, B. M. (2007). Acquisition of nicotine self-administration in adolescent rats given prolonged access to the drug. Neuropsychopharmacology, 32(3), 700–709.

Chen, J. C., Das, B., Mead, E. L., & Borzekowski, D. L. G. (2017). Flavored E-cigarette Use and Cigarette Smoking Susceptibility among Youth. Tobacco Regulatory Science, 3(1), 68–80.

Collins, G. T., Newman, A. H., Grundt, P., Rice, K. C., Husbands, S. M., Chauvignac, C., Chen, J., Wang, S., & Woods, J. H. (2007). Yawning and hypothermia in rats: effects of dopamine D3 and D2 agonists and antagonists. Psychopharmacology, 193(2), 159–170.

Collins, G. T., Witkin, J. M., Newman, A. H., Svensson, K. A., Grundt, P., Cao, J., & Woods, J. H. (2005). Dopamine agonist-induced yawning in rats: a dopamine D3 receptor-mediated behavior. The Journal of pharmacology and experimental therapeutics, 314(1), 310–319.

Cooper, S. Y., Akers, A. T., & Henderson, B. J. (2021). Flavors Enhance Nicotine Vapor Self-administration in Male Mice. Nicotine & Tobacco Research, 23(3), 566–572.

Correa, V.L., Flores, R.J., Carcoba, L.M., Arreguin, M.C. & O'Dell, L.E. (2019). Sex differences in cholinergic systems in the interpeduncular nucleus following nicotine exposure and withdrawal. Neuropharmacology, 158, 107714.

Covington, H. E., 3rd, & Miczek, K. A. (2003). Vocalizations during withdrawal from opiates and cocaine: possible expressions of affective distress. European journal of pharmacology, 467(1-3), 1–13.

D'Aquila, P. S., Rossi, R., Rizzi, A., & Galistu, A. (2012). Possible role of dopamine D1-like and

D2-like receptors in behavioural activation and "contingent" reward evaluation in sodium-replete and sodium-depleted rats licking for NaCl solutions. Pharmacology, biochemistry, and behavior, 101(1), 99–106.

Dawkins, L., Turner, J., Roberts, A., & Soar, K. (2013). 'Vaping' profiles and preferences: an online survey of electronic cigarette users. Addiction (Abingdon, England), 108(6), 1115–1125.

Drope, J., Cahn, Z., Kennedy, R., Liber, A. C., Stoklosa, M., Henson, R., Douglas, C. E., & Drope, J. (2017). Key issues surrounding the health impacts of electronic nicotine delivery systems (ENDS) and other sources of nicotine. CA: A Cancer Journal for Clinicians, 67(6), 449–471.

Ervin, G. N., Schmitz, S. A., Nemeroff, C. B., & Prange, A. J., Jr (1981). Thyrotropin-releasing hormone and amphetamine produce different patterns of behavioral excitation in rats. European journal of pharmacology, 72(1), 35–43.

Etter, J. F. (2016). A longitudinal study of cotinine in long-term daily users of e-cigarettes. Drug and Alcohol Dependence, 160, 218-221.

FDA. Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke. Food and Drug Administration, 2012.

Flores, R. J., Alshbool, F. Z., Giner, P., O'Dell, L. E., & Mendez, I. A. (2022). Exposure to Nicotine Vapor Produced by an Electronic Nicotine Delivery System Causes Short-Term Increases in Impulsive Choice in Adult Male Rats. Nicotine & Tobacco Research, 24(3), 358–365.

Flores, R. J., Cruz, B., Uribe, K. P., Correa, V. L., Arreguin, M. C., Carcoba, L. M., Mendez, I.

A., & O'Dell, L. E. (2020). Estradiol promotes and progesterone reduces anxiety-like behavior produced by nicotine withdrawal in female rats. Psychoneuroendocrinology, 119, 104694.

Flores, R. J., Uribe, K. P., Swalve, N., & O'Dell, L. E. (2019). Sex differences in nicotine intravenous self-administration: A meta-analytic review. Physiology & Behavior, 203, 42–50.

Flouris, A. D., Chorti, M. S., Poulianiti, K. P., Jamurtas, A. Z., Kostikas, K., Tzatzarakis, M. N., Wallace Hayes, A., Tsatsakis, A. M., & Koutedakis, Y. (2013). Acute impact of active and passive electronic cigarette smoking on serum cotinine and lung function. Inhalation Toxicology, 25(2), 91–101.

Frie, J.A., Underhill, J., Zhao, B., De Guglielmo, G., Tyndale, R.F., & Khokhar, J.Y. (2020). OpenVape: An open-source e-cigarette vapor exposure device for rodents. Eneuro, 7(5), ENEURO.0279–20.

Galistu, A., & D'Aquila, P. S. (2013). Dopamine on D2-like receptors "reboosts" dopamine D1-like receptor-mediated behavioural activation in rats licking for a isotonic NaCl solution. Psychopharmacology, 229(2), 357–366.

George, O., Grieder, T. E., Cole, M., & Koob, G. F. (2010). Exposure to chronic intermittent nicotine vapor induces nicotine dependence. Pharmacology Biochemistry and Behavior, 96(1), 104–107.

George, O., and Koob, G. F., (2017). Overview of Nicotine Withdrawal and Negative Reinforcement (Preclinical). In Negative Affective States and Cognitive Impairments in Nicotine Dependence (pp. 1–20). Elsevier. Academic Press.

Gilpin, N. W., Whitaker, A. M., Baynes, B., Abdel, A. Y., Weil, M. T., & George, O. (2014).

Nicotine vapor inhalation escalates nicotine self-administration. Addiction Biology, 19(4), 587–592.

Giner, P., Maynez-Anchondo, L., Liley, A. E., Uribe, K. P., Frietze, G. A., Simon, N. W., & Mendez, I. A. (2022). Increased Risky Choice and Reduced CHRNB2 Expression in Adult Male Rats Exposed to Nicotine Vapor. International journal of molecular sciences, 23(3), 1231.

Hamilton, K. R., Berger, S. S., Perry, M. E., & Grunberg, N. E. (2009). Behavioral effects of nicotine withdrawal in adult male and female rats. Pharmacology, biochemistry, and behavior, 92(1), 51–59.

Hatsukami, D., LaBounty, L., Hughes, J., Laine, D., (1993). Effects of tobacco abstinence on food intake among cigarette smokers. Health Psychology, 12(6), 499. Hecht, S. S., (2012). Lung carcinogenesis by tobacco smoke. International Journal of Cancer, 131(12), 2724–2732.

Heishma, S. J., Taylor, R. C., Henningfield, J. E., (1994). Nicotine and smoking: a review of effects on human performance. Experimental and Clinical Psychopharmacology, 2(4), 345.

Heishman S. J. Kleykamp B. A. and Singleton E. G. (2010) Meta-analysis of the acute effects of nicotine and smoking on human performance. Psychopharmacology (Berl). 210, 453–469.

Hogle, J. M., and Curtin, J. J., (2006). Sex differences in negative affective response during nicotine withdrawal. Psychophysiology, 43(4), 344–356.

Hughes, J. R., (1992). Tobacco withdrawal in self-quitters. Journal of Consulting and Clinical Psychology, 60(5), 689.

Hughes, J. R., (2007). Measurement of the effects of abstinence from tobacco: a qualitative review. Psychology of Addictive Behaviors, 21(2), 127.

Hukkanen, J., Jacob P. and Benowitz N. L. (2005) Metabolism and disposition kinetics of nicotine. Pharmacol Rev. 57, 79–115.

Jackson, K. J., Muldoon, P. P., De Biasi, M., & Damaj, M. I. (2015). New mechanisms and perspectives in nicotine withdrawal. Neuropharmacology, 96, 223–234.

Javadi-Paydar, M., Kerr, T. M., Harvey, E. L., Cole, M., & Taffe, M. A. (2019). Effects of nicotine and THC vapor inhalation administered by an electronic nicotine delivery system (ENDS) in male rats. Drug and Alcohol Dependence, 198, 54–62.

Kallupi, M., de Guglielmo, G., Larrosa, E. & George, O. (2019). Exposure to passive nicotine vapor in male adolescent rats produces a withdrawal-like state and facilitates nicotine self-administration during adulthood. European Neuropsychopharmacology, 29(11), 1227-1234.

Karson, C. N., Staub, R. A., Kleinman, J. E., & Wyatt, R. J. (1981). Drug effect on blink rates in rhesus monkeys: preliminary studies. Biological psychiatry, 16(3), 249–254.

Kaye, S., Gilsenan, J., Young, J. T., Carruthers, S., Allsop, S., Degenhardt, L., van den Brink, W. (2014). Risk behaviours among substance use disorder treatment seekers with and without adult ADHD symptoms. Drug and Alcohol Dependence, 144, 70–77.

Khan, H. A., Urstadt, K. R., Mostovoi, N. A., & Berridge, K. C. (2020). Mapping excessive "disgust" in the brain: Ventral pallidum inactivation recruits distributed circuitry to make sweetness "disgusting". Cognitive, affective & behavioral neuroscience, 20(1), 141–159.

King, B. A., Patel, R., Nguyen, K. H., & Dube, S. R. (2015). Trends in Awareness and Use of

Electronic Cigarettes Among US Adults, 2010-2013. Nicotine & Tobacco Research, 17(2), 219–227.

Klenowski, P.M., Zhao-Shea, R., Freels, T.G., Molas, S., & Tapper, A.R. (2021). Dynamic activity of interpeduncular nucleus GABAergic neurons controls expression of nicotine withdrawal in male mice. Neuropsychopharmacology, 47(3), 641–651.

Kong, G., & Krishnan-Sarin, S. (2017). A call to end the epidemic of adolescent E-cigarette use. Drug and Alcohol Dependence, 174, 215–221.

Lallai, V., Chen, Y. C., Roybal, M. M., Kotha, E. R., Fowler, J. P., Staben, A., Cortez, A., & Fowler, C. D. (2021). Nicotine e-cigarette vapor inhalation and self-administration in a rodent model: Sex- and nicotine delivery-specific effects on metabolism and behavior. Addiction biology, 26(6), e13024.

Le Foll B. and Goldberg S. R. (2009). Effects of nicotine in experimental animals and humans: an update on addictive properties. Hand Exp Pharmacol. 192, 335–367.

Lefever, T. W., Lee, Y. O. K., Kovach, A. L., Silinski, M. A. R., Marusich, J. A., Thomas, B. F., & Wiley, J. L. (2017). Delivery of nicotine aerosol to mice via a modified electronic cigarette device. Drug and Alcohol Dependence, 172, 80–87.

Lefever, T. W., Thomas, B. F., Kovach, A. L., Snyder, R. W., & Wiley, J. L. (2019). Route of administration effects on nicotine discrimination in female and male mice. Drug and Alcohol Dependence, 204, 107504.

Levin, E. D., Lawrence, S. S., Petro, A., Horton, K., Rezvani, A. H., Seidler, F. J., & Slotkin, T. A. (2007). Adolescent vs. adult-onset nicotine self-administration in male rats: Duration of effect and differential nicotinic receptor correlates. Neurotoxicology and Teratology, 29(4), 458–465.

Matta, S. G., Balfour, D. J., Benowitz, N. L., Boyd, R. T., Buccafusco, J. J., Caggiula, A. R., Craig, C. R., Collins, A. C., Damaj, M. I., Donny, E. C., Gardiner, P. S., Grady, S. R., Heberlein, U., Leonard, S. S., Levin, E. D., Lukas, R. J., Markou, A., Marks, M. J., McCallum, S. E., Parameswaran, N., … Zirger, J. M. (2007). Guidelines on nicotine dose selection for in vivo research. Psychopharmacology, 190(3), 269–319.

Miliano, C., Scott, E. R., Murdaugh, L. B., Gnatowski, E. R., Faunce, C. L., Anderson, M. S., Reyes, M. M., Gregus, A. M., & Buczynski, M. W. (2020). Modeling drug exposure in rodents using e-cigarettes and other electronic nicotine delivery systems. Journal of neuroscience methods, 330, 108458.

Montanari, C., Kelley, L. K., Kerr, T. M., Cole, M., & Gilpin, N. W. (2020). Nicotine e-cigarette vapor inhalation effects on nicotine & cotinine plasma levels and somatic withdrawal signs in adult male Wistar rats. Psychopharmacology, 237(3), 613–625.

Natividad, L. A., Torres, O. V., Friedman, T. C., & O'Dell, L. E. (2013). Adolescence is a period of development characterized by short- and long-term vulnerability to the rewarding effects of nicotine and reduced sensitivity to the anorectic effects of this drug. Behavioural brain research, 257, 275–285.

National Research Council. (2010). Guide for the care and use of laboratory animals. National Academies Press. https://scholar.google.com/scholar_lookup?title=Guide+for+the+care+and+use+of+laboratory+animals&publication_year=2010&

O'Dell L. E. (2009). A psychobiological framework of the substrates that mediate nicotine use during adolescence. Neuropharmacology, 56 Suppl 1(Suppl 1), 263–278.

O'Dell, L. E., Bruijnzeel, A. W., Ghozland, S., Markou, A., & Koob, G. F. (2004). Nicotine

withdrawal in adolescent and adult rats. Annals of the New York Academy of Sciences, 1021, 167–174.

O'Dell, L. E., Bruijnzeel, A. W., Smith, R. T., Parsons, L. H., Merves, M. L., Goldberger, B. A., Richardson, H. N., Koob, G. F., & Markou, A. (2006). Diminished nicotine withdrawal in adolescent rats: implications for vulnerability to addiction. Psychopharmacology, 186(4), 612–619.

O'Dell, L. E., Chen, S. A., Smith, R. T., Specio, S. E., Balster, R. L., Paterson, N. E., Markou, A., Zorrilla, E. P., & Koob, G. F. (2007). Extended access to nicotine self-administration leads to dependence: Circadian measures, withdrawal measures, and extinction behavior in rats. The Journal of pharmacology and experimental therapeutics, 320(1), 180–193.

O'Dell, L. E., & Khroyan, T. V. (2009). Rodent models of nicotine reward: what do they tell us about tobacco abuse in humans?. Pharmacology, biochemistry, and behavior, 91(4), 481–488.

O'Dell, L. E., & Torres, O. V. (2014). A mechanistic hypothesis of the factors that enhance vulnerability to nicotine use in females. Neuropharmacology, 76 Pt B(0 0), 566–580.

Pang, R. D., Goldenson, N. I., Kirkpatrick, M., Barrington-Trimis, J. L., Cho, J. & Leventhal, A. M. (2020). Sex differences in the appeal of flavored e-cigarettes among young adult e-cigarette users. Psychology Addiction Behavior, 34(2), 303-307.

Paton, J. F., Abdala, A. P., Koizumi, H., Smith, J. C., & St-John, W. M. (2006). Respiratory rhythm generation during gasping depends on persistent sodium current. Nature neuroscience, 9(3), 311–313.

Paton, J. F., & St-John, W. M. (2007). Counterpoint: Medullary pacemaker neurons are essential

for gasping, but not eupnea, in mammals. Journal of applied physiology (Bethesda, Md. : 1985), 103(2), 718–722.

Patten, T., Dreier, A., Herman, R. J., Kimball, B. A., & De Biasi, M. (2021). Exposure to fruit-flavoring during adolescence increases nicotine consumption and promotes dose escalation. Neuropharmacology, 195, 108672.

Pauly, J. R., (2008). Gender differences in tobacco smoking dynamics and the neuropharmacological actions of nicotine. Front Biosci, 13(1), 505–516.

Pepper, J. K., & Brewer, N. T. (2014). Electronic nicotine delivery system (electronic cigarette) awareness, use, reactions, and beliefs: a systematic review. Tobacco Control, 23(5), 375–384.

Perkins, K. A., Briski, J., Fonte, C., Scott, J., Lerman, C., (2009). Severity of tobacco abstinence symptoms varies by time of day. Nicotine and Tobacco Research, 11(1), 84–91

Perkins, K. A., Levine, M., Marcus, M., Shiffman, S., D'Amico, D., Miller, A., Keins, A., Ashcom, J., & Broge, M. (2000). Tobacco withdrawal in women and menstrual cycle phase. Journal of consulting and clinical psychology, 68(1), 176–180.

Piñeiro, B., Correa, J. B., Simmons, V. N., Harrell, P. T., Menzie, N. S., Unrod, M., Meltzer, L. R., & Brandon, T. H. (2016). Gender differences in use and expectancies of e-cigarettes: Online survey results. Addictive behaviors, 52, 91–97.

Piper, M. E., Schlam, T. R., Cook, J. W., Sheffer, M. A., Smith, S. S., Loh, W.-Y., Hefner, K. R., (2011). Tobacco withdrawal components and their relations with cessation success. Psychopharmacology, 216(4), 569–578.

Pogocki, D., Ruman, T., Danilczuk, M., Danilczuk, M., Celuch, M., & Wałajtys-Rode, E.

(2007). Application of nicotine enantiomers, derivatives and analogues in therapy of neurodegenerative disorders. European journal of pharmacology, 563(1-3), 18–39.

Pomerleau, C. S., and Pomerleau, O. F., (1992). Euphoriant effects of nicotine in smokers. Psychopharmacology, 108(4), 460–465.

Pontieri, F. E., Tanda, G., Orzi, F., & Di Chiara, G. (1996). Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. Nature, 382(6588), 255–257.

Ponzoni, L., Moretti, M., Sala, M., Fasoli, F., Mucchietto, V., Lucini, V., Cannazza, G., Gallesi, G., Castellana, C. N., Clementi, F., Zoli, M., Gotti, C. & Braida, D. (2015). Different physiological and behavioural effects of e-cigarette vapour and cigarette smoke in mice. European Neuropsychopharmacology, 25(10), 1775-1786.

Schassburger, R. L., Pitzer, E. M., Smith, T. T., Rupprecht, L. E., Thiels, E., Donny, E. C., & Sved, A. F. (2016). Adolescent Rats Self-Administer Less Nicotine Than Adults at Low Doses. Nicotine & Tobacco Research, 18(9), 1861–1868.

Schmid, B., Hohm, E., Blomeyer, D., Zimmermann, U. S., Schmidt, M. H., Esser, G., & Laucht, M. (2007). Concurrent alcohol and tobacco use during early adolescence characterizes a group at risk. Alcohol and alcoholism (Oxford, Oxfordshire), 42(3), 219–225.

Shiffman, S., and Sayette, M. A., (2005). Validation of the nicotine dependence syndrome scale (NDSS): a criterion-group design contrasting chippers and regular smokers. Drug and Alcohol Dependence, 79(1), 45–52.

Singh, V. P., Jain, N. K., & Kulkarni, S. K. (2001). On the antinociceptive effect of fluoxetine, a selective serotonin reuptake inhibitor. Brain research, 915(2), 218–226.

Smith, L.C., Kallupi, M., Tieu, L., Shankar, K., Jaquish, A., Barr, J., Su, Y., Velarde, N.,

Sedighim, S., Carrette, L.L.G., Klodnicki, M., Sun, X., De Guglielmo, G., & George, O. (2020). Validation of a nicotine vapor self-administration model in rats with relevance to electronic cigarette use. Neuropsychopharmacology, 45(11), 1909–1919.

Stringfield, S. J., Madayag, A. C., Boettiger, C. A. & Robinson, D. L. (2019). Sex differences in nicotine-enhanced Pavlovian conditioned approach in rats. Biology of Sex Differences, 10(1), 37.

Tedeschi, D. H., Fowler, P. J., Fujita, T., & Miller, R. B. (1967). Mechanisms underlying reserpine-induced ptosis and blepharospasm: evidence that reserpine decreases central sympathetic outflow in rats. Life sciences, 6(5), 515–523. https://doi.org/10.1016/0024-3205(67)90055-0

Trauth, J. A., Seidler, F. J., & Slotkin, T. A. (2000). Persistent and delayed behavioral changes after nicotine treatment in adolescent rats. Brain research, 880(1-2), 167–172.

Torres, O., Tejeda, H., Natividad, L., & O'Dell, L.E. (2008). Enhanced vulnerability to the rewarding effects of nicotine during the adolescent period of development. Pharmacology Biochemistry and Behavior, 90(4), 658–663.

Torres, O. V., Natividad, L. A., Tejeda, H. A., Van Weelden, S. A., & O'Dell, L. E. (2009). Female rats display dose-dependent differences to the rewarding and aversive effects of nicotine in an age-, hormone-, and sex-dependent manner. Psychopharmacology, 206(2), 303–312.

Torres, O. V., Gentil, L. G., Natividad, L. A., Carcoba, L. M. & O'Dell, L.E. (2013). Behavioral, biochemical, and molecular indices of stress are enhanced in female versus male rats experiencing nicotine withdrawal. Frontiers in Psychiatry, 4, 38.

Torres, O. V., Pipkin, J. A., Ferree, P., Carcoba, L. M., & O'Dell, L. E. (2015). Nicotine

withdrawal increases stress-associated genes in the nucleus accumbens of female rats in a hormone-dependent manner. Nicotine & Tobacco Research, 17(4), 422–430.

U.S. Department of Health and Human Services., (2014). The Health Consequences of Smoking—50 Years of Progress. A Report of the Surgeon General. Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health.

World Health Organization. (2011). WHO Report on the Global Tobacco Epidemic.

World Health Organization. (2017). WHO Report on the Global Tobacco Epidemic.

Xu, X., Shrestha, S. S., Trivers, K. F., Neff, L., Armour, B. S., & King, B. A. (2021). U.S. healthcare spending attributable to cigarette smoking in 2014. Preventive medicine, 150, 106529.

Yoong, S. L., Stockings, E., Chai, L. K., Tzelepis, F., Wiggers, J., Oldmeadow, C., Paul, C., Peruga, A., Kingsland, M., Attia, J., & Wolfenden, L. (2019). Prevalence of electronic nicotine delivery systems (ENDS) use among youth globally: A systematic review and meta-analysis of country level data. Australian and New Zealand Journal of Public Health, 42(3), 303–308.

Zhu, S.H., Gamst, A., Lee, M., Cummins, S., Yin, L., & Zoref, L. (2013). The Use and Perception of Electronic Cigarettes and Snus among the U.S. Population. PLOS ONE, 8(10), e79332.

Table 1. Individual Physical Signs of Withdrawal

Physical Signs of Withdrawal	Definition and Modulatory Systems	Citations
Blinks	Rapid opening and closing of both eyes associated with nicotine withdrawal. Elicited by dopamine D1 agonist administration.	O'Dell et al. (2004) Karson et al. (1981)
Yawns	Opening of the mouth widely. Associated with alcohol and opioid withdrawal. Elicited by dopamine D3 receptor activation.	Collins et al. (2005) Collins et al. (2007)
Teeth chatters	Rapid chattering of the teeth. Associated with alcohol and opioid withdrawal. Elicited by thyrotropic releasing hormone in the hypothalamus.	Ervin et al. (1981)
Gasps	Rapid and audible inhale and exhale. Associated with opioid and nicotine withdrawal. Modulated by brainstem systems in the pons and medulla.	Paton et al. (2006) Paton & St. John (2007)
Writhes	Contraction of abdominal muscles that move up the body. Associated with nicotine and opioid withdrawal. Regulated via serotonergic and opioid systems.	Singh et al. (2001)
Body shakes	Shaking of the body up to the shoulders. Associated with alcohol and opioid withdrawal. Elicited by the thyrotropic releasing hormone in the hypothalamus.	Ervin et al. (1981)
Head shakes	Shaking of the head. Associated with alcohol and nicotine withdrawal. Elicited by serotonin 2A receptor activation.	Bedard & Pycock (1977)
Ptosis	Half closing of both eyelids and is usually a symptom of malaise. Associated with opioid and nicotine withdrawal. Elicited by inhibition of monoamine release.	Tedeschi et al. (1967)
Grooming	Licking/washing of the forepaws, face, and/or body. Purported to reflect self-soothing during nicotine withdrawal. Associated with GABA neuron activation in the interpeduncular nucleus.	Klenowski et al. (2021)
Licks	Tongue extends and retracts on the body and/or surfaces. Associated with liking and motivated behavior. Elicited by dopamine D1 and D2 receptor activation.	D'Aquila et al. (2012) Galistu & D'Aquila (2013)

Table 2. Statistical Analyses.

Figure	Panel	Test	Dependent Variables	Independent Variables	Comparison	Statistical value	p-value	Effect size
1	Day 1	3-way ANOVA	Nosepokes	Age Sex Treatment	Age	$F(1,40) = 8.74$	$p = 0.005$*	$\eta^2 = 0.18$
					Sex	$F(1,40) = 0.01$	$p = 0.91$	$\eta^2 < 0.001$
					Treatment	$F(1,40) = 13.08$	$p < 0.001$*	$\eta^2 = 0.25$
					Age x Sex	$F(1,40) = 1.24$	$p = 0.27$	$\eta^2 = 0.03$
					Age x Treatment	$F(1,40) = 0.58$	$p = 0.45$	$\eta^2 = 0.01$
					Sex x Treatment	$F(1,40) = 0.15$	$p = 0.70$	$\eta^2 = 0.004$
					Age x Sex x Treatment	$F(1,40) = 0.46$	$p = 0.50$	$\eta^2 = 0.01$
1	Day 14	3-way ANOVA	Nosepokes	Age Sex Treatment	Age x Sex	$F(1,40) = 17.80$	$p < 0.001$*	$\eta^2 = 0.31$
					Age x Treatment	$F(1,40) = 5.61$	$p = 0.02$*	$\eta^2 = 0.12$
					Sex x Treatment	$F(1,40) = 12.61$	$p < 0.001$*	$\eta^2 = 0.24$
					Age x Sex x Treatment	$F(1,40) = 1.76$	$p = 0.19$	$\eta^2 = 0.04$
2	Adolescents	3-way ANOVA	Nosepokes % change from Day 1	Time Sex Treatment	Time x Sex x Treatment	$F(1,40) = 8.68$	$p = 0.005$*	$\eta^2 = 0.18$
2	Adults	3-way ANOVA	Nosepokes % change from Day 1	Time Sex Treatment	Time x Sex x Treatment	$F(1,20) = 17.83$	$p < 0.001$*	$\eta^2 = 0.31$
3A	Adolescents & Adults	3-way ANOVA	Total Physical Signs	Age Sex Treatment	Age x Sex	$F(1,40) = 0.05$	$p = 0.82$	$\eta^2 = 0.001$
					Age x Treatment	$F(1,40) = 9.24$	$p = 0.004$*	$\eta^2 = 0.19$
					Sex x Treatment	$F(1,40) = 1.11$	$p = 0.30$	$\eta^2 = 0.03$
					Age x Sex x Treatment	$F(1,40) = 0.05$	$p = 0.82$	$\eta^2 = 0.001$
3B	Adolescents & Adults	3-way ANOVA	Teeth Chatters	Age Sex Treatment	Age	$F(1,40) = 0.58$	$p = 0.45$	$\eta^2 = 0.01$
					Sex	$F(1,40) = 1.30$	$p = 0.26$	$\eta^2 = 0.03$
					Treatment	$F(1,40) = 13.08$	$p < 0.001$*	$\eta^2 = 0.25$
					Age x Sex	$F(1,40) = 1.30$	$p = 0.26$	$\eta^2 = 0.03$
					Age x Treatment	$F(1,40) = 0.91$	$p = 0.35$	$\eta^2 = 0.02$
					Sex x Treatment	$F(1,40) = 1.78$	$p = 0.19$	$\eta^2 = 0.04$
					Age x Sex x Treatment	$F(1,40) = 0.91$	$p = 0.35$	$\eta^2 = 0.02$
3C	Adolescents & Adults	3-way ANOVA	Blinks	Age Sex Treatment	Age	$F(1,40) = 9.64$	$p = 0.003$*	$\eta^2 = 0.19$
					Sex	$F(1,40) = 0.11$	$p = 0.74$	$\eta^2 = 0.003$
					Treatment	$F(1,40) = 103.87$	$p < 0.001$*	$\eta^2 = 0.72$
					Age x Sex	$F(1,40) = 0.30$	$p = 0.59$	$\eta^2 = 0.01$
					Age x Treatment	$F(1,40) = 0.01$	$p = 0.91$	$\eta^2 < 0.001$
					Sex x Treatment	$F(1,40) = 0.83$	$p = 0.37$	$\eta^2 = 0.02$
					Age x Sex x Treatment	$F(1,40) = 0.16$	$p = 0.69$	$\eta^2 = 0.004$
3D	Adolescents & Adults	3-way ANOVA	Grooming	Age Sex Treatment	Age x Sex	$F(1,40) = 0.22$	$p = 0.64$	$\eta^2 = 0.01$
					Age x Treatment	$F(1,40) = 24.68$	$p < 0.001$*	$\eta^2 = 0.38$
					Sex x Treatment	$F(1,40) = 1.22$	$p = 0.28$	$\eta^2 = 0.03$
					Age x Sex x Treatment	$F(1,40) = 0.40$	$p = 0.53$	$\eta^2 = 0.01$
4	Adolescents & Adults	3-way ANOVA	% time spent in closed arm	Age Sex Treatment	Age	$F(1,40) = 0.14$	$p = 0.71$	$\eta^2 = 0.003$
					Sex	$F(1,40) = 3.07$	$p = 0.09$	$\eta^2 = 0.07$
					Treatment	$F(1,40) = 3.20$	$p = 0.08$	$\eta^2 = 0.07$
					Age x Sex	$F(1,40) = 0.25$	$p = 0.62$	$\eta^2 = 0.01$
					Age x Treatment	$F(1,40) = 1.06$	$p = 0.31$	$\eta^2 = 0.03$
					Sex x Treatment	$F(1,40) = 0.001$	$p = 0.98$	$\eta^2 < 0.001$
					Age x Sex x Treatment	$F(1,40) = 0.05$	$p = 0.82$	$\eta^2 = 0.001$
5A	Adolescents	Pearson's correlation	Nosepokes vs Physical Signs	Sex Treatment	Control Females	$r = -0.66$	$p = 0.16$	---
					Control Males	$r = 0.41$	$p = 0.41$	---
					Nicotine Females	$r = 0.25$	$p = 0.64$	---
					Nicotine Males	$r = -0.13$	$p = 0.81$	---
5B	Adults	Pearson's correlation	Nosepokes vs Physical Signs	Sex Treatment	Control Females	$r = 0.07$	$p = 0.90$	---
					Control Males	$r = -0.15$	$p = 0.78$	---
					Nicotine Females	$r = -0.11$	$p = 0.84$	---
					Nicotine Males	$r = -0.13$	$p = 0.81$	---
5C	Adolescents	Pearson's correlation	Nosepokes vs % time spent in closed arm	Sex Treatment	Control Females	$r = 0.20$	$p = 0.71$	---
					Control Males	$r = 0.17$	$p = 0.75$	---
					Nicotine Females	$r = 0.80$	$p = 0.05$*	---
					Nicotine Males	$r = -0.58$	$p = 0.22$	---
5D	Adults	Pearson's correlation	Nosepokes vs % time spent in closed arm	Sex Treatment	Control Females	$r = -0.27$	$p = 0.61$	---
					Control Males	$r = -0.36$	$p = 0.48$	---
					Nicotine Females	$r = 0.59$	$p = 0.21$	---
					Nicotine Males	$r = 0.08$	$p = 0.88$	---
6	Adolescents	3-way ANOVA	Body Weight % change from Day 1	Time Sex Treatment	Time x Sex	$F(1,40) = 6.63$	$p = 0.01$*	$\eta^2 = 0.14$
					Time x Treatment	$F(1,40) = 0.08$	$p = 0.78$	$\eta^2 = 0.002$
					Sex x Treatment	$F(1,40) = 0.01$	$p = 0.92$	$\eta^2 < 0.001$
					Time x Sex x Treatment	$F(1,40) = 0.01$	$p = 0.92$	$\eta^2 < 0.001$
6	Adults	3-way ANOVA	Body Weight	Time Sex	Time	$F(1,40) = 5.77$	$p = 0.02$*	$\eta^2 = 0.13$
					Sex	$F(1,40) = 0.81$	$p = 0.38$	$\eta^2 = 0.02$

			% change from Day 1	Treatment	Treatment Time x Sex Time x Treatment Sex x Treatment Time x Sex x Treatment	$F(1,40) = 0.36$ $F(1,40) = 0.80$ $F(1,40) = 0.37$ $F(1,40) = 0.21$ $F(1,40) = 0.21$	$p = 0.55$ $p = 0.38$ $p = 0.55$ $p = 0.65$ $p = 0.65$	$\eta^2 = 0.01$ $\eta^2 = 0.02$ $\eta^2 = 0.01$ $\eta^2 = 0.01$ $\eta^2 = 0.01$
7	Adolescents & Adults	3-way ANOVA	Cotinine levels	Age Sex Treatment	Age x Sex x Treatment	$F(1,40) = 8.94$	$p = 0.005$*	$\eta^2 = 0.18$

Figure 1. The data reflect mean (± SEM) nosepoke responses in a port that delivered nicotine vapor or ambient air (control) in female and male adolescent and adult rats on Day 1 and Day 14 of the exposure regimen. Individual data points are color matched to allow for comparisons in each pair of rats. The asterisks (*) denote a significant difference from controls, the daggers (†) denote a difference from males, and the at sign (@) denotes a difference from adults ($p \leq 0.05$).

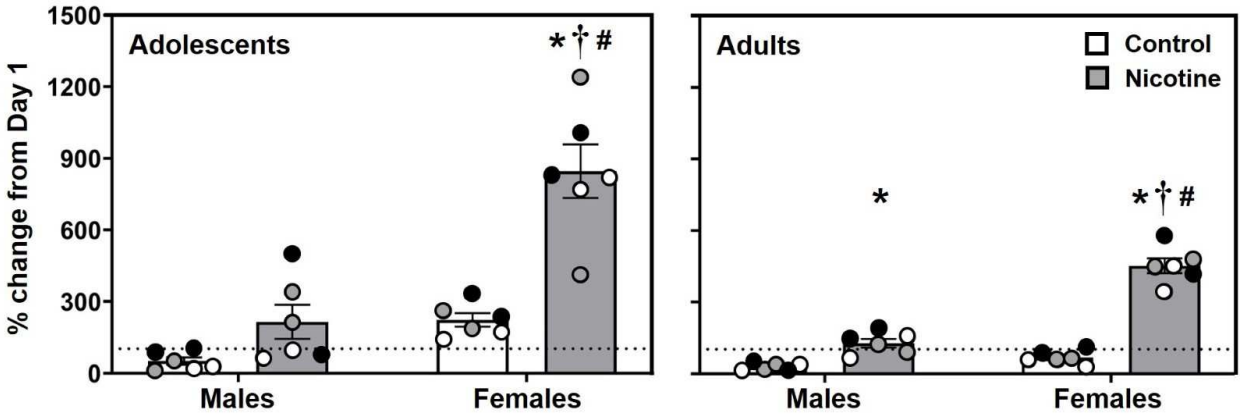


Figure 2. The data reflect mean (± SEM) nosepoke responses on Day 14 expressed as % change from Day 1 in female and male adolescent and adult rats. Individual data points are color matched to allow for comparisons in each pair of rats. The asterisks (*) denote a significant difference from controls, the daggers (†) denote a difference from males, and the number signs (#) denote a difference from Day 1 of the exposure regimen ($p \leq 0.05$).

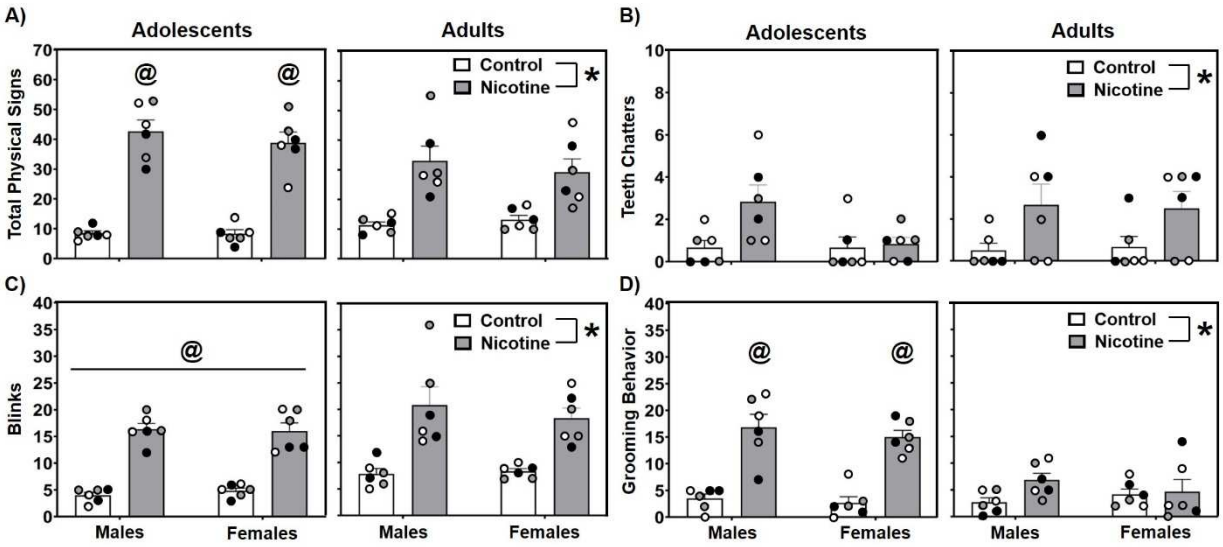


Figure 3. The data reflect mean (± SEM) total physical signs (A), teeth chatters (B), blinks (C), and grooming (D) in female and male adolescent and adult rats on Day 14 of the exposure regimen following precipitated withdrawal. The asterisks (*) denote a significant difference from controls and the at sign (@) denotes a difference from adults ($p<0.05$).

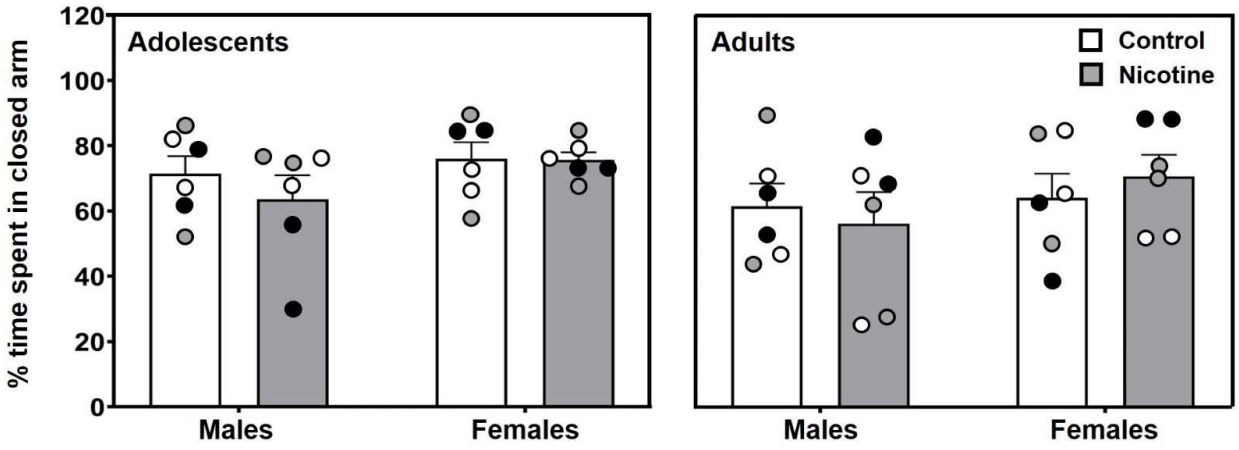


Figure 4. The data reflect mean (± SEM) anxiety-like behavior on Day 14 expressed as % time spent in the closed arm of the EPM in female and male adolescent and adult rats.

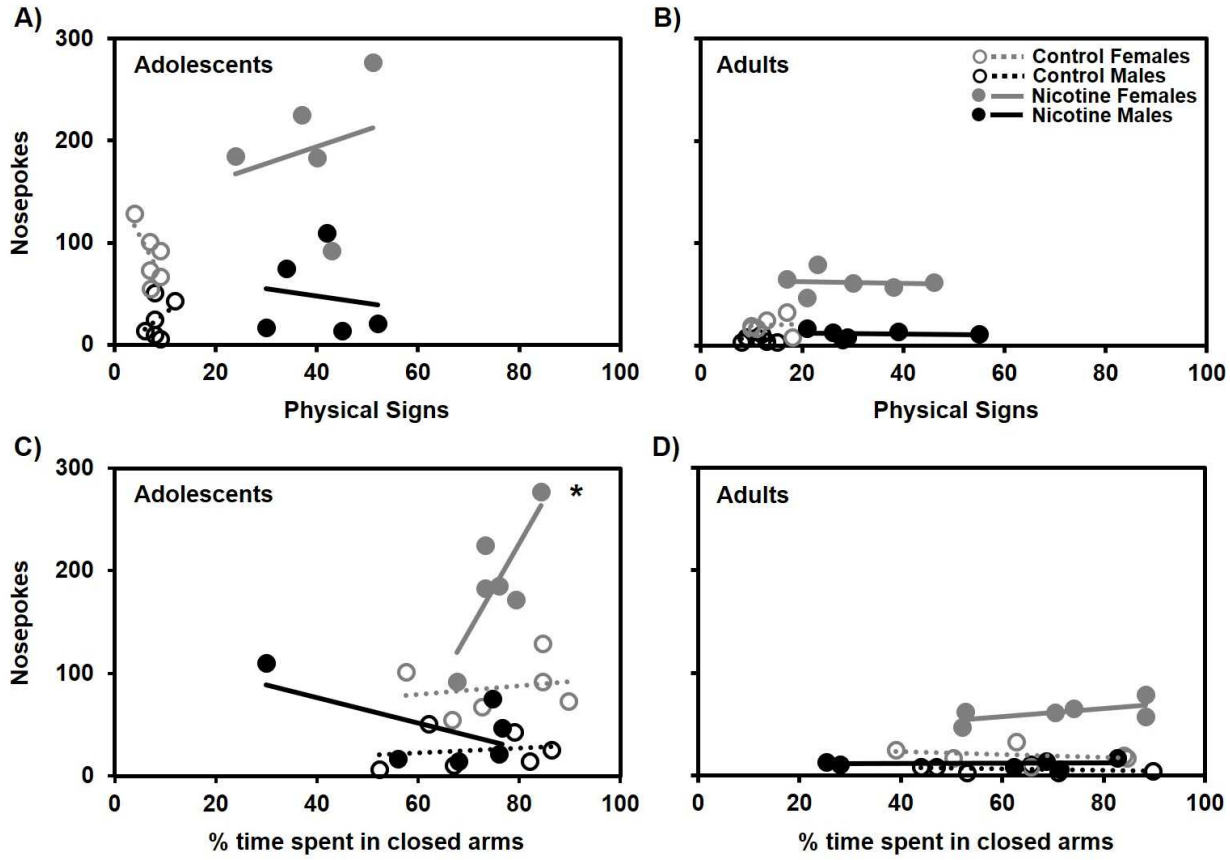


Figure 5. The data reflect a correlational analysis between nosepoke responses and withdrawal-induced increases in physical signs and anxiety-like behavior, which is noted as % time spent in the closed arm of the EPM on Day 14. The asterisk (*) denotes a significant correlation between nosepoke responses and % time spent in the closed arm of the EPM ($p \leq 0.05$).

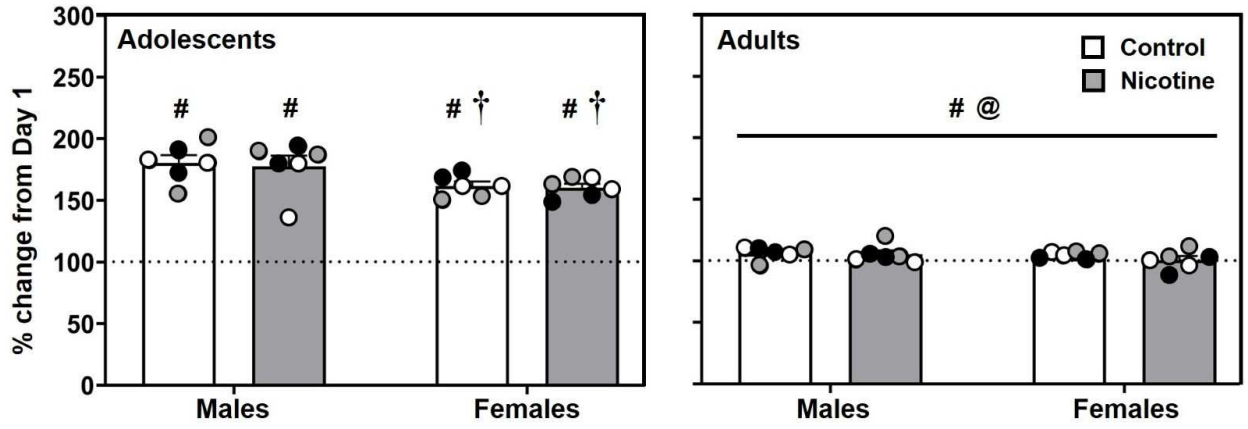


Figure 6. The data reflect mean (± SEM) changes in body weight on Day 14 expressed as % change from Day 1 in female and male adolescent and adult rats. Individual data points are color matched to allow for comparisons in each pair of rats. The daggers (†) denote a significant difference from males, the at sign (@) denotes a difference from adults, and the number signs (#) denote a difference from Day 1 ($p \leq 0.05$).

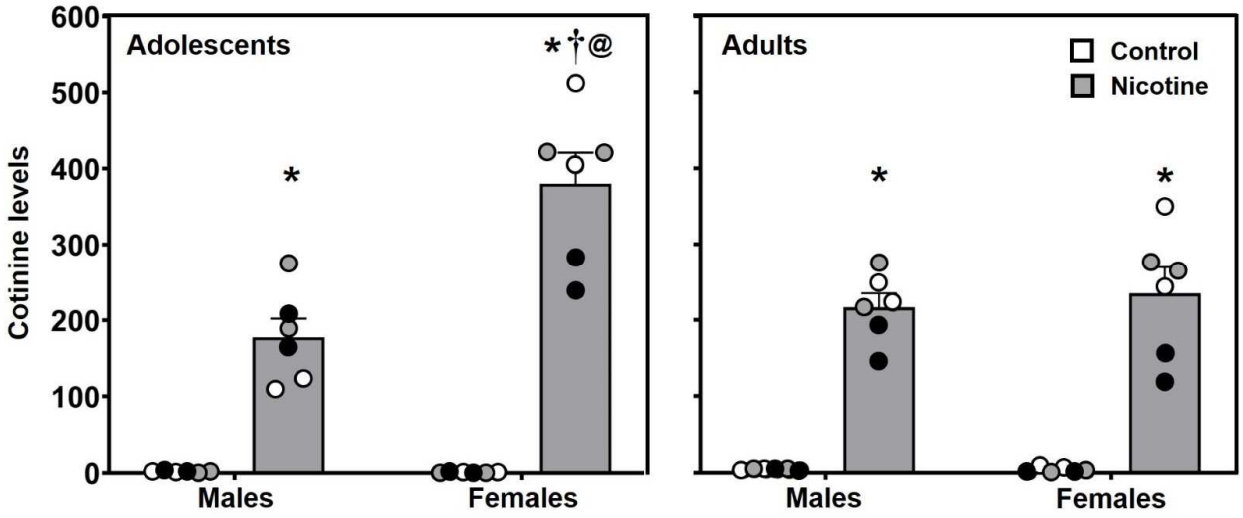


Figure 7. The data reflects mean (± SEM) serum cotinine levels in female and male adolescent and adult rats on Day 14 of the exposure regimen. Individual data points are color matched to allow for comparisons in each pair of rats. The asterisks (*) denote a significant difference from controls, the dagger (†) denotes a difference from males, and the at sign (@) denotes a difference from adults ($p \leq 0.05$).

Vita

Veronika Evangelina Espinoza was born and raised in Escondido, California. In 2018, she completed her Bachelor's degree in Biochemistry and received two minors in Psychology and Mathematics at the California State University of San Marcos. Veronika became interested in Behavioral Neuroscience research as an undergraduate and began conducting research in the laboratory of Dr. Keith A. Trujillo. Her research focused on the locomotor effects of amphetamine-like stimulants in adolescent and adult rodents. Afterwards, she worked as a post-baccalaureate student at The Scripps Research Institute in La Jolla, California in the Neuroscience laboratory of Dr. Olivier George. There, her research focused on early life exposure mimicking clinical use of oxycodone in adolescents and how that altered drug-seeking and escalation behavior during adulthood. In August of 2019, Veronika joined the Doctoral Program in Psychology at The University of Texas at El Paso under the mentorship of Dr. Laura E. O'Dell. Her research interests include understanding the mechanisms that promote age and sex differences in the behavioral effects of nicotine withdrawal. Veronika graduated with her Master's degree in Experimental Psychology in May of 2022. She had 10 oral and 19 poster abstracts accepted at numerous scientific conferences. She received the *National Award of Excellence in Research* by a graduate student from the National Hispanic Science Network and a fellowship award from the *Enhanced* Interdisciplinary Research Training Institute (*e*IRTI) at the University of Southern California. Veronika has published 1 first-author article and co-authored 1 publication in peer-reviewed journals such as *Drug and Alcohol Dependence* and the *Journal of the Experimental Analysis of Behavior*.

Contact information: vespinoza12@miners.utep.edu

This Master's Thesis was typed by Veronika Evangelina Espinoza.