


The role of β -Nicotyrine in E-Cigarette abuse liability I: Drug Discrimination

Smethells, JR^{1,2}, Wilde, S¹, Muelken, P¹, LeSage, MG^{1,2,3,4}, Harris, AP^{1,2,3}

¹Hennepin Healthcare Research Institute, Minneapolis, MN, USA

²Department of Medicine, University of Minnesota, Minneapolis, MN, USA

³Department of Psychology, University of Minnesota, Minneapolis, MN, USA

⁴Department of Pharmacology, University of Minnesota, Minneapolis, MN, USA

 Correspondence should be addressed to:

John Smethells, Ph.D.
Hennepin Healthcare Research Institute
701 Park Ave
Minneapolis, MN 55415
E-mail: smet0011@umn.edu

Abstract

Background: β -Nicotyrine (β -Nic) is a unique minor alkaloid constituent in electronic nicotine delivery systems (ENDS) that is derived from nicotine (Nic) degradation and can reach 25% of Nic concentrations in ENDS aerosol. β -Nic slows Nic metabolism and prolongs systemic Nic exposure, which may alter the discriminability of Nic. The present study sought to examine β -Nic has interoceptive effects itself, and if it alters the subjective effects ENDS products within a drug-discrimination paradigm. *Methods:* The pharmacodynamics of β -Nic were examined in vitro, and a nicotine discrimination paradigm was used to determine if β -Nic (0 – 5.0 mg/kg) shares discriminative stimulus properties with Nic (0.2 mg/kg) in male (n = 13) and female (n = 14) rats after 10- & 60-min β -Nic pretreatment delays. A second group of rats was trained to discriminate β -Nic and Nornicotine (Nornic) from saline to determine if β -Nic alone has interoceptive properties and whether they are similar to Nornic. *Results:* β -Nic had similar binding affinity and efficacy at the $\alpha 4\beta 2$ nicotinic receptor subtype as Nornic, ~50% of Nic efficacy. However, β -Nic only weakly substituted for Nic during substitution testing in female rats, but not males, whereas Nornic fully substituted for Nic. Combination testing at the 10 and 60-min pretreatment intervals showed that β -Nic dose-dependently increased the duration of nicotine's discriminative stimulus effects, especially at the 60-min delay. Drug naïve rats could reliably discriminate Nornic, but not β -Nic, from Sal. *Conclusion:* β -Nic increased and prolonged the interoceptive stimulus properties of Nic, suggesting it may alter to the abuse liability of ENDS through its ability to slow Nic metabolism.

1. Introduction

The 2022 Monitoring the Future Survey found that a greater percentage of high school seniors report past 30-day use of electronic nicotine delivery systems (ENDS) compared to cigarettes (20.7% vs 4.0%, respectively; Meich, et. al., 2022). This is in stark contrast to 2011 when the past 30-day use of ENDS was vastly overshadowed by combustible cigarettes (1.5% vs 15.8%, respectively; Arrazola et al., 2015). Thus, ENDS are now primarily responsible for establishing nicotine (Nic) dependence in adolescents and understanding what product characteristics of ENDS contribute to its abuse liability is critical to inform tobacco regulatory policy.

Nic is the primary constituent underlying the abuse liability of tobacco products (Benowitz, 2010; Benowitz et al., 2016), yet their addictive properties cannot be solely attributed to Nic alone. Several studies have shown that smokers who switch from conventional tobacco cigarettes (e.g., 15 mg Nic) to very-low Nic content cigarettes (VLNC; e.g., 0.5 mg Nic or %0.33 of full flavor) still maintain considerable smoking levels (15-20 VLNCs/day; Benowitz et al., 2012; Donny et al., 2007, 2015), although more recent studies show lower rates of smoking VLNCs when alternative nicotine products (e.g. ENDS) are available (Hatsukami et al., 2024). The continued use of VLNCs could be due, in part, to non-Nic constituents that are present in VLNC smoke (e.g, Nornic, acetaldehyde, β -carbolines; Won Weymarn et al., 2016). Such compounds can mimic or enhance the central nervous system (CNS) effects of Nic and/or have abuse liability themselves (Bardo et al., 1999; Benowitz et al., 2016; Caggiula et al., 2009; LeSage et al., 2018). As such, they are considered by the FDA CTP to be addiction-related harmful or potentially harmful constituents” (HPHCs; Hoffman and Evans, 2013). This designation by the FDA identifies constituents whose levels should be monitored and potentially limited to reduce the toxicity and addictiveness of tobacco products. However, this list was last updated in 2012 before ENDS rose in popularity. Given that ENDS heat a liquid rather than ignite tobacco, there is a large disparity in the chemical profile of ENDS aerosol vs combustible

tobacco products. For example, β -nicotyrine (β -Nic) can be substantially more prevalent in ENDS aerosol (~50x or 25% of Nic levels) than in tobacco smoke (Jacob et al., 1999; Martinez et al., 2015; Son et al., 2018). This is a concern because β -Nic is a potent inhibitor of the hepatic enzyme cytochrome P450 (2A6 & 2A13) and slows Nic metabolism both *in vitro* (Denton et al., 2004; Kramlinger et al., 2012; Shigenaga et al., 1989) and *in vivo* (Stålhandske & Slanina, 1982). This work prompted a nicotyrine hypothesis (Abramovitz et al., 2015) that it should modulate ENDS abuse liability in humans.

β -Nic is formed in ENDS through two processes: 1) dehydrogenation during ENDS aerosolization (Clayton et al., 2009) and 2) oxidation as ENDS liquid ages (Martinez et al., 2015; Wada et al., 1959). Dehydrogenation occurs during the pyrolysis (i.e., heating) of Nic-containing e-liquid solutions. Specifically, the greatest level of thermal degradation of Nic into β -Nic occurs at 200-550°C (Clayton et al., 2009; Liu et al., 1999; Woodward & Haines, 1944), which is in the temperature range (215-475°C) that an ENDS coil reaches to aerosolize e-liquids (Flora et al., 2017; Talih et al., 2019). Studies from our lab (Harris et al., 2019) and by others (Martinez et al., 2015; Son et al., 2018) have found that the aerosol produced by fresh e-liquids can contain β -Nic concentrations up to ~13% of Nic levels, with the range varying from 1.1% to 12.9% depending on the heating temperature, ratio of propylene glycol to vegetable glycerin (the ENDS delivery vehicle), Nic concentration, vaping topography, and other factors (see Son et al., 2018). Oxidation occurs as an ENDS liquid ages, which can be accelerated by periodically opening and resealing solutions (i.e., “steeping”; a common practice among ENDS users with refillable tanks [Zhan et al., 2017]). After several weeks, concentrations of β -nic increase to 8% of Nic levels in non-aerosolized solutions and can reach 25% of Nic levels in ENDS aerosols (Martinez et al., 2015). These upper levels are ~1000x higher than the concentration of β -Nic in combustible cigarettes which burn at ~700-900°C (Trehy et al., 2011), a temperature far outside

of the ideal range for β -Nic formation. Given its prevalence and potential to influence ENDS abuse liability, further research that directly tests the β -Nic hypothesis is needed.

Sex differences may also influence establishment of ENDS dependence. Clinical and preclinical research consistently finds that females exceed males across all phases of the Nic use disorder process, with women having more difficulty quitting smoking in adulthood (K. A. Perkins, 2001; K. A. Perkins et al., 2000; Sofuoglu et al., 1999) perhaps due to increased Nic cue reactivity (K. A. Perkins, 2001), menstrual hormone cycling (progesterone vs. estrogen) and pharmacokinetic factors (Nic metabolism is slower in women; Benowitz & Hatsukami 1998; Carroll et al., 2004; Carroll and Lynch, 2016; Carroll and Smethells, 2016; Lynch et al., 2002; Perkins et al., 1999; Pogun et al., 2017). In line with the epidemiological research, preclinical research has often found that female rats acquire quicker and maintain higher Nic self-administration (NSA) than males, especially at lower doses (Caggiula et al., 2001; Donny et al., 2000; Grebenstein et al., 2013; Li et al., 2014; Lynch, 2009; Rezvani et al., 2008; Swalve et al., 2016a; Wang et al., 2014) and that they self-administer more than males under extended access 23 hour-sessions (vs. 1-2 hr short-access ones; Flores et al., 2019). As such, the present study sought to characterize the impact of β -Nic on the abuse liability of Nic in both sexes.

The present study used a drug discrimination paradigm to determine if Nic and β -Nic produce similar interoceptive stimulus properties and if they enhance the discriminability of one another when combined (i.e., whether they feel alike and whether the combination contributes to similar CNS effects to make either drug more discriminable; see Stolerman et al., 1984). In this model, rats are reinforced with food for appropriately responding on a Nic- or Sal-associated lever following a pre-session injection of a training dose of Nic (e.g., 0.2 mg/kg) or Sal, respectively (i.e., the injection type indicates which lever produces food reinforcers). Drugs of the same class, such as Nornic and other nAChR agonists, can enhance the interoceptive

effects of Nic and cause a leftward shift in the Nic dose-response curve (Desai et al., 2016; Stolerman, 1993). One of the primary neurobiological mediators of Nic discrimination is $\alpha 4\beta 2$ nAChR agonism (Smith & Stolerman, 2009), and thus we also examined if β -Nic, like Nornic, has binding affinity and efficacy at this receptor subtype. If it does, it is hypothesized that like Nornic (Caine et al., 2014; Goldberg et al., 1989), β -Nic may also partially substitute for Nic during substitution testing. Regardless, the ability of β -Nic to slow nicotine metabolism is expected to prolong the half-life of Nic when they are combined and thereby shift the Nic discrimination dose-response curve to the left (Desai et al., 2016; but see Caine et al., 2014), especially under longer pretreatment intervals (10 vs 60-mins).

2. Method

2.1. Animals

Male and female (275g and 225g at delivery, respectively) Sprague-Dawley (SD; Envigo, Madison, WI) rats were individually housed with free access to chow and water in a temperature- (22° C) and humidity-controlled colony room. One week after arrival, rats were food restricted and given 16g or 18g of food per day for female and male rats, respectively. Protocols were approved by the Hennepin Healthcare Research Institute's Institutional Animal Care and Use Committee and were in accordance with NIH guidelines set forth in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

2.2. Apparatus

Drug discrimination operant chambers (Med-Associates, St. Albans, VT) were composed of aluminum and polycarbonate walls and a stainless-steel grid floor. The chamber had two response levers on the front panel, each with a white stimulus light located directly above and a food hopper between the levers (food pellets: BioServ #F0021), and the back panel contained a house light mounted centrally at the top. Chambers were contained in sound-attenuating boxes

equipped with ventilation fans that provided masking noise. A computer (OS: Windows 10[®]) running MED-PC IV[®] (Med Associates) orchestrated experimental sessions and recorded data.

2.3. Drugs

(-) Nicotine base (Sigma Chemical Co., St. Louis, MO) was dissolved into saline to form a 30 mg/ml stock solution verified by gas chromatography with nitrogen phosphorous detection using our routine assay (LeSage et al., 2003). Solutions varied by no more than $\pm 5\%$ (average $< 1\%$) from the target concentration. β -Nic (obtained as a *neat oil* from Cayman Chemical, Ann Arbor, MI) was diluted to 50 mg/ml in pure EtOH. All drug doses, including Sal, were diluted in Sal and 6% v/v EtOH (w/v = 0.79g/ml; a non-behaviorally active *i.p.* dose of 39.5 mg/kg; ED₅₀ of EtOH for drug discrimination is 450-560 mg/kg, see Bienkowski & Kostowski, 1998) to obtain target doses administered at 1ml/kg. The addition of 6% EtOH to Sal and all drug combinations was done to control for the highest EtOH concentration used in the final study in this series (3.0 mg/ml β -Nic *iv* self-administration, 1% higher than necessary for the present study); however, note that during substitution testing a higher 5.0 mg/kg dose was employed (i.e., 10% EtOH v/v), however, given the lack of behavioral effects at this dose the entire substitution dose-effect curve was not redetermined with this higher EtOH concentration. All drug doses are expressed as the base.

2.4. Procedure

2.4.1. *Ki determinations, receptor binding profiles and agonist/antagonist function of Nic, β -Nic and Nornic.* Assays were conducted by the NIMH Psychoactive Drug Screening Program (PDSP), which uses human embryonic kidney cells that stably express functional nAChR subtypes to examine the relative difference in binding affinity (assays were limited to: $\alpha 2\beta 2$, $\alpha 2\beta 4$, $\alpha 3\beta 2$, $\alpha 3\beta 4$, $\alpha 4\beta 2$, $\alpha 4\beta 4$ and $\alpha 7$) and functional agonist/antagonist efficacy (assays were limited to: $\alpha 4\beta 2$ & $\alpha 3\beta 4$) of Nic, β -nic and Nornic (10 mg/ml samples of each drug were

submitted for analysis). The assay protocols are provided in greater detail within the PDSP assay protocol book (Roth, 2018).

2.4.2.1. *Nic discrimination training.* Rats ($n = 16$ per sex; 2 females and 3 males were lost due to health issues) were initially trained (Mon-Fri) to respond in an operant chamber on two levers to earn sucrose pellets during sessions using our previously established methods (Harris et al., 2012; LeSage et al., 2012, 2009); sessions ended following 30 min or 50 reinforcers, whichever occurred first. The terminal schedule was a variable interval (VI) 15 sec schedule (for each lever, a response after an average of 15 sec produced a sucrose pellet). The active lever was alternated every other session during initial training. Once responding was sufficient on each lever (< 40 pellets per session), Nic/Sal discrimination training commenced. Specifically, rats were injected s.c. with Nic (0.2 mg/kg) or Sal (Sal; 1ml/kg) and then immediately placed in the operant chamber for a 10-min blackout prior to the start of the session. Once the training session started, as indicated by the house and cue lights illuminating, rats received sucrose reinforcers for responding on one lever if Nic was administered or the other lever if Sal was administered, with the Nic lever assignment counterbalanced across rats. Injections followed a two-session alternation between Nic and Sal (i.e., Nic, Nic, Sal, Sal, Nic, Nic, etc.). Acquisition of the Nic/Sal discrimination was assessed twice weekly (Tue & Fri) during 2-min extinction test sessions wherein no food reinforcers were delivered for Nic/Sal-appropriate responses and then the session continued normally by reinforcing drug-appropriate level responses. Once response rates and Nic discrimination during training and test sessions were stable ($> 95\%$ or $> 80\%$ injection-appropriate responding across 4 consecutive training or extinction test sessions, respectively, and no trend in overall response rate), substitution and combination testing occurred.

2.4.2.2. *Substitution testing and combination testing across 10- and 60-min (combination only) pretreatment intervals.* Substitution and combination testing was conducted twice weekly

(Tue/Fri) provided behavior was stable on intervening Nic training days (see 2.4.1.1). Following the 2-min extinction test session, rats were reinforced for responding on either lever as neither lever was drug-appropriate; the exceptions were the 0.0 and 0.2 Nic doses, which were run as normal training sessions. All **substitution testing** involved s.c. pretreatment 10 min prior to the session with Nic (0.0, 0.0125, 0.025, 0.05, 0.1, or 0.2 mg/kg), Nornic and β -Nic (0.0, 0.01, 0.025, 0.1, 0.25, 1.0, 2.5 and 5.0 mg/kg for both; drug order counterbalanced). The dose ranges of Nic and Nornic were based on prior studies showing that they bracket the range of discriminable doses (Caine et al., 2014; Stolerman et al., 2011). The lower β -Nic doses were selected to be vaping-relevant concentrations (e.g., 5 - 25% of the Nic doses used for training; Martinez et al., 2015), whereas the higher doses were based on the aforementioned Nornic drug discrimination dose range given the similarity of Nornic and β -Nic in our pharmacokinetic binding and affinity work (Fig. 1). After substitution testing, **combination testing** commenced, wherein Nic (0.0, [0.0125 10-min only], 0.025, 0.05, 0.1, & 0.2 mg/kg) discrimination was examined when combined with a low, moderate, and high dose of β -Nic (0, 0.05, 0.25, & 1.0 mg/kg, respectively) under both a 10- and 60-min drug pretreatment delay prior to the start of the session (the drug mixture was administered at 1mg/ml). The goal of the longer pretreatment interval was to assess if the ability of β -Nic to slow Nic metabolism and prolong the discriminative stimulus effects Nic. All rats were initially tested with the Nic + 0.25 mg/kg β -Nic under the 10- and 60-min pretreatment times, and then subsequent Nic + β -Nic conditions (i.e., Nic + 0.0, + 0.05 & + 1.0 mg/kg β -Nic) were counterbalanced across subjects at one pretreatment interval and then repeated at the other pretreatment interval. The Nic alone condition under the 10-min pretreatment used the data from the initial Nic dose response during substitution testing. The lower β -Nic dose (0.05 mg/kg) was chosen as it is 25% of the training Nic dose, which provided a vaping-relevant β -Nic concentrations found in ENDS vapor.

2.4.2.3 *β -Nic and Nornic discrimination training and testing.* A separate group of rats (N = 15: 8 male, 7 female) underwent drug discrimination training using both a 2 and then a 5 mg/kg training dose of β -Nic and Nornic using the same training and testing protocol described in 2.4.1.1. Once lever press training criteria were met (reliably earning 50 pellets over 30 minutes), rats were given sc injections of Sal or drug 10-min prior to each 30-min session. Following two weeks of discrimination training, test sessions were conducted twice weekly (Tue/Fri) with training sessions continuing normally throughout this period. Rats experienced the following order of test sessions (min. 5 test sessions and until visually stable) that assessed discrimination between Sal (1ml/kg; sc) vs 2 mg/kg β -Nic (10 sessions), 2 mg/kg Nornic (10 sessions), 5 mg/kg β -Nic (10 sessions), 5 mg/kg Nornic (20 sessions), 5 mg/kg β -Nic (5 sessions).

2.5 Data Analysis

2.5.1. *Drug Discrimination.* Only the data from the 2-min extinction test sessions were analyzed. The primary dependent measures were the percentage of responses on the Nic-associated lever (%NLR) and the response rate during each 2-min test session, to assess general motoric effects. The data were assessed using a mixed-model two-way ANOVA (Dose x Sex) within each drug tested and were followed by Tukey post-hoc tests to compare each drug dose to Sal, and Sidak post-hoc tests to compare drugs at each dose. Full substitution was defined as %NLR greater than or equal to 80%, while partial substitution was defined as 20%-80% NLR. During combination testing (both at 10- and 60-min pretreatment conditions) a three-way (sex x β -Nic dose x Nic dose) ANOVA was conducted and upon a significant main effect or interaction with sex, then a separate two-way ANOVA was conducted within each sex to determine the effect of the Nic + β -Nic combination across nicotine dose. Post-hoc analyses used a Geisser-Greenhouse correction when necessary. Data analysis was conducted in Prism v.10.2.3 and R v. 4.3.2.

3. Results

3.1.1. *Ki determinations, receptor binding profiles and agonist/antagonist function of Nic, β -Nic and Nornic.* The inhibition constant for β -Nic was higher at the $\alpha 4\beta 2$ nAChR subtype (K_i : 103.9 nM) compared to nicotine (K_i : 6.096 nM, Fig. 1a), but was somewhat similar to Nornic (K_i : 69.94 nM), indicating its affinity at the $\alpha 4\beta 2$ nAChR subtype is lower than nicotine and more similar to Nornic. The functional assays found that β -Nic had similar agonist properties as Nornic (Fig. 1b; i.e., they produced similar $^{86}\text{Rb}^+$ efflux). Like Nornic, β -Nic did not inhibit nicotine's agonism at the $\alpha 4\beta 2$ nAChR subtype (Fig. 1c; i.e., neither produced a significant change [i.e. inhibition] in nicotine induced $^{86}\text{Rb}^+$ efflux). Efficacy at the $\alpha 3\beta 4$ subtype was low (9% of Nic agonism & 20% inhibition of Nic induced $^{86}\text{Rb}^+$ efflux; data not shown). Lastly, binding affinity (K_i in nM) of Nicotine, Nornic, and β -Nic was also conducted at $\alpha 2\beta 2$ - (Nic: 4.0; Nornic: 23.9; β -nic: 132.5), $\alpha 2\beta 4$ - (Nic: 6.4; Nornic: 84.5; β -nic: 173.7), $\alpha 3\beta 2$ - (Nic: 8.9; Nornic: 25.4; β -nic: 216.6), $\alpha 3\beta 4$ - (Nic: 1.95; Nornic: 2.17; β -nic: 0.6), $\alpha 4\beta 4$ - (Nic: 24.3; Nornic: 4.8; β -nic: 5.7) and $\alpha 7$ -containing (Nic: 6.4; Nornic: 84.5; β -nic: 173.7) nAChR subtypes, with generally weaker affinity except at $\alpha 3\beta 4$ and $\alpha 4\beta 4$.

3.2.1. *Nicotine dose-effect curve and substitution profile of β -Nic & Nornic.* Figure 2 plots the %NLR and response rate for Nic, Nornic and β -Nic pretreatment 10 min prior to the test sessions. The Nic dose-effect curve showed a significant main effect of Nic dose ($F_{3,31, 72.88} = 102.3, p < 0.001$), but no sex or sex x dose interaction, and response rates showed a sex ($F_{1,25} = 7.330, p < 0.05$) and a sex x dose interaction ($F_{5,125} = 6.911, p < 0.001$). Analysis of the β -Nic dose-effect substitution profile found a significant main effect of β -Nic dose ($F_{7,175} = 5.105, p < 0.001$), with only females showing significantly higher %NLR at moderate β -Nic doses vs. Sal. There was a main effect of sex ($F_{1,25} = 8.360, p < 0.01$) on response rate for β -Nic, but no significant within-sex effects. For Nornic (i.e., the positive control) there was a significant effect

of dose ($F_{7,175} = 60.68$, $p < 0.001$) and dose x sex interaction ($F_{7,175} = 2.507$, $p < 0.05$) on %NLR, and a main effect of dose ($F_{3,35,83.62} = 3.854$, $p < 0.01$) and sex ($F_{1,25} = 4.745$, $p < 0.05$) on response rates. Post-hoc tests revealed significant differences from Sal at higher doses for both %NLR and response rate (See Figure 2).

3.2.2. Combination testing of Nic and β -Nic with a 10-min pretreatment interval. Figure 3 plots the %NLR for combinations of Nic + 0.05, 0.25 & 1.0 β -Nic 10 min prior to the test session. An overall analysis of the conditions found a significant effect of Sex ($F_{1,25} = 7.98$, $p < 0.01$), Nic dose ($F_{5,125} = 418.25$, $p < 0.001$), and interactions of Sex x Nic dose ($F_{5,125} = 3.37$, $p < 0.01$) and Nic dose x β -Nic dose ($F_{15,375} = 2.04$, $p < 0.05$). Analysis of the Nic alone condition found a main effect of Nic dose ($F_{3,53,88.19} = 118.8$, $p < 0.001$), but not sex, indicating a similar ability to discriminate Nic from saline in both sexes. At the clinically relevant 0.05 mg/kg β -Nic dose, there were significant main effects of Nic dose ($F_{5,125} = 203.7$, $p < 0.001$), β -Nic ($F_{1,25} = 5.539$, $p < 0.05$) and Sex ($F_{1,25} = 5.600$, $p < 0.05$). In females, there was a main effect of β -Nic ($F_{1,13} = 5.420$, $p < 0.05$), however, post-hoc tests did not show %NLR to differ at any Nic dose. At the moderate 0.25 mg/kg β -Nic dose, there was a main effect of Nic dose ($F_{5,125} = 201.3$, $p < 0.001$), Sex ($F_{1,25} = 2.507$, $p < 0.01$) and an interaction of Nic dose x Sex ($F_{5,125} = 2.332$, $p < 0.05$), but no effect of β -Nic; follow-up tests found no significant main effect of β -Nic in either sex. Lastly, at the the highest β -Nic dose, there was a main effect of Nic dose ($F_{5,125} = 262.7$, $p < 0.001$), β -Nic dose ($F_{0.76,18.98} = 5.428$, $p < 0.05$), Sex ($F_{1,25} = 2.507$, $p < 0.01$) and interactions of Nic dose x Sex ($F_{5,125} = 2.365$, $p < 0.05$) and Nic dose x β -Nic ($F_{3,453,86.34} = 3.202$, $p < 0.05$). Within-sex analyses revealed a main effect of β -Nic in males ($F_{1,12} = 4.950$, $p < 0.05$) and an interaction β -Nic x Nic dose in females ($F_{5,65} = 2.587$, $p < 0.05$), with the combination producing significantly higher %NLR vs Nic alone at 0.1 mg/kg Nic in males and 0.05 mg/kg Nic in females (see bottom panels in Figure 3).

3.2.3. *Combination testing of Nic and β -Nic with a 60-min pretreatment interval.* Figure 4 displays the %NLR during the 2-min test probes that occurred 60-minutes post-injection. An overall analysis of the conditions found a significant effect of Nic dose ($F_{4, 100} = 146.5, p < 0.001$), β -Nic dose ($F_{3, 75} = 6.59, p < 0.001$), and interactions of Sex x Nic dose ($F_{4, 100} = 3.30, p < 0.05$), Nic dose x β -Nic dose ($F_{12, 300} = 4.80, p < 0.001$) and lastly a Sex x Nic Dose x β -Nic dose ($F_{12, 300} = 2.00, p < 0.05$). In the follow-up analysis of the Nic alone condition, there was a significant sex difference in the ability to discriminate the interoceptive stimuli of Nic after 60 min with females showing higher %NLR than males, especially at higher doses. Analysis of the Nic Alone discrimination curves found a main effect of Nic dose ($F_{2.97, 74.13} = 27.91, p < 0.001$), sex ($F_{1, 25} = 6.412, p < 0.05$) and an interaction of Nic dose and sex ($F_{4, 100} = 4.491, p < 0.01$). Post-hoc tests revealed a significant difference at the 0.2 mg/kg dose. Analysis of the combination testing at the clinically relevant dose, Nic + 0.05 mg/kg β -Nic tended to show greater %NLR 60 min post injection compared to Nic alone. Analysis revealed a main effect of Nic dose ($F_{4, 100} = 73.04, p < 0.001$), β -Nic ($F_{1, 25} = 7.002, p < 0.05$), Nic dose x β -Nic ($F_{4, 100} = 9.673, p < 0.001$) and sex x Nic dose ($F_{4, 100} = 4.094, p < 0.05$); a follow-up two-way ANOVA in males and females found an interaction between Nic dose x β -Nic ($F_{4, 96} = 7.002, p < 0.05$ & $F_{4, 96} = 3.597, p < 0.01$, respectively) with both sexes showing significant differences from Nic alone at the 0.02 Nic + β -Nic combination dose in a post-hoc tests. Combination testing at a moderate dose of β -Nic, Nic + 0.25 mg/kg β -Nic, also found greater %NLR 60 min post injection compared to Nic alone as evidenced by a main effect of Nic dose ($F_{4, 100} = 71.75, p < 0.001$), β -Nic ($F_{1, 25} = 8.614, p < 0.01$) and an interaction of Nic dose x β -Nic ($F_{4, 100} = 5.974, p < 0.001$), β -Nic x sex ($F_{1, 25} = 8.125, p < 0.01$). A follow-up test in males found a main effect of β -Nic ($F_{1, 12} = 15.05, p < 0.01$) and an interaction of Nic dose x β -Nic ($F_{4, 48} = 9.722, p < 0.001$) with a significant difference from Nic alone at the 0.02 Nic + 0.25 β -Nic mg/kg combination dose. Analysis of the highest combination dose of β -Nic (Nic + 1.0 mg/kg β -Nic) found a main effect of Nic dose ($F_{4, 100} = 83.76, p < 0.001$),

sex ($F_{1,25} = 7.354$, $p < 0.05$), β -Nic ($F_{0.80,20} = 21.76$, $p < 0.001$) and an interaction of Nic dose x β -Nic ($F_{3.05,76.30} = 11.32$, $p < 0.001$), Nic dose x sex ($F_{4,100} = 4.374$, $p < 0.001$). Follow-up tests in males found a main effect of β -Nic ($F_{1,24} = 7.813$, $p < 0.01$), and an interaction of Nic x β -Nic dose ($F_{4,96} = 6.524$, $p < 0.001$), whereas in females an interaction of Nic x β -Nic dose ($F_{4,104} = 4.415$, $p < 0.001$) was found; post-hoc tests indicated a significant differences from Nic alone in both sexes at the 0.1 and 0.2 Nic + β -Nic combination doses.

Figure 5 presents response rates during the drug discrimination sessions at both the 10- and 60-min pretreatment intervals. An overall assessment of the 10-min pretreatment condition found a main effect of Sex ($F_{1,25} = 8.58$, $p < 0.01$) and β -Nic dose ($F_{3,75} = 3.29$, $p < 0.05$), and an interaction of Sex x Nic dose ($F_{4,100} = 12.13$, $p < 0.001$) and Nic dose x β -Nic dose ($F_{15,375} = 2.04$, $p < 0.05$). To assess Sex x Nic dose interaction, response rates were assessed in both sexes. During the 10-min pretreatment condition, there were no significant differences in males, however, females showed a main effect of Nic dose ($F_{2.57,33.40} = 17.17$, $p < 0.001$) on response rates across β -Nic doses (Figure 5; left column), with significantly higher response rates at the two highest Nic concentrations within each β -Nic dose. Given there was no main effect of β -Nic dose in either sex, response rates were collapsed across β -Nic dose to examine sex differences in response rates. A main effect of Sex ($F_{1,30} = 11.45$, $p < 0.01$) and Nic dose x Sex interaction ($F_{5,150} = 9.855$, $p < 0.001$) were observed with significant differences between sexes at all but the two highest Nic doses. An overall assessment of the 60-min pretreatment condition found a main effect of Sex ($F_{1,25} = 5.42$, $p < 0.05$) and β -Nic dose ($F_{3,75} = 5.67$, $p < 0.001$), and an interaction of Sex x Nic dose ($F_{4,100} = 5.87$, $p < 0.001$) and Sex x β -Nic dose ($F_{3,75} = 3.24$, $p < 0.001$). To assess Sex x Nic dose interaction, response rates were assessed in both sexes. In males, there was a significant main effect of β -Nic dose ($F_{2.03,24.35} = 6.365$, $p < 0.01$), but no significant differences from Nic alone were found during post-hoc tests. Females (Figure 5; right column) showed a main effect of Nic dose ($F_{2.50,32.46} = 8.002$, $p < 0.01$) across β -Nic doses, with

significantly higher response rates at the highest three Nic concentrations within Nic alone dose-effect.

3.3. Acquisition of β -Nic and Nornic drug discrimination. Figure 6 – left panel - shows that rats were unable to discriminate the interoceptive effects of β -Nic from Sal during discrimination testing, however, some rats were able to discriminate Nornic from Sal at both the 2 and 5 mg/kg doses (Figure 6 – right panel). Specifically, a *t*-test comparing B-Nic vs Nornic used the average of the final 3 sessions of each condition and found Nornic discrimination was only significantly higher than β -Nic at the 5 mg/kg doses ($t_{14} = 7.17$) after a Bonferroni correction ($p < 0.025$).

4. Discussion

The results of this study indicate that β -Nic prolongs the discriminative interoceptive properties of Nic in rats within a drug discrimination paradigm. The primary mechanism underlying this effect is presumed to mostly be due to the ability of β -Nic to significantly slow Nic metabolism, as evidenced by the leftward shift in the dose-effect curve during the 60-min pretreatment interval (Fig. 4). A second possible mechanism is that β -Nic has direct CNS effects, as evidenced by the PD binding/affinity at $\alpha 4\beta 2$ nAChRs that was similar to Nornic, (Fig. 1), the ability for it to weakly substitute for Nic during substitution testing in females (Fig. 2), and the slightly better Nic discrimination at moderate doses when combined with β -Nic at the 10-min pretreatment interval (Fig. 3). However, the inability for β -Nic to serve as a discriminative stimulus in Nic naïve rats (Fig. 6) and the inability of it to substitute for Nic in male rats (Fig. 2), contradicts the notion that the direct CNS effects of β -Nic played a role in moderating Nic's discriminative stimulus effects. Regardless of the mechanisms involved, the present work found that clinically relevant doses of β -Nic increase the ability of rats to discriminate Nic and may therefore contribute to the abuse liability of ENDS products.

4.1. *Pharmacodynamics of β -Nic*. The binding affinity and efficacy of β -Nic (Fig. 1) was akin to Nornic at the $\alpha 4\beta 2$ nAChR. These findings are intriguing because the $\alpha 4\beta 2$ subtype is the primary neurobiological mediator of nicotine's discriminative, ICSS and reinforcing effects (Ator and Griffiths, 2003; Benowitz, 2010; Smith and Stolerman, 2009). Thus, it is surprising that β -Nic did not exhibit discriminative stimulus effects similar to Nornic and will be discussed in more detail below.

4.2. *Substitution for Nic by β -Nic and Nornic*. The present study found that Nornic almost completely substituted for Nic in both male and female rats (Figure 2), which replicates previous studies showing that Nornic has interoceptive stimulus effects similar to Nic (Caine et al., 2014; Goldberg et al., 1989). β -Nic, however, only weakly substituted for Nic in female rats, suggesting it may have weak Nic-like interoceptive properties. This finding is somewhat surprising, given that the binding and functional effects were similar to Nornic and it is presently unclear why β -Nic had no behavioral effects when administered alone.

4.3. *Combination testing under 10- and 60-min pretreatment intervals*. Combinations of Nic and β -Nic caused Nic to be more discriminable at moderate nicotine doses (Figure 3). At the 10-min pretreatment interval, this effect on Nic discrimination at moderate Nic doses was dependent on the β -Nic dose, with both sexes showing significant enhancement at the 1.0 m/kg β -Nic dose. While it is unclear whether this effect was due to the direct CNS effects of β -Nic or the ability of β -Nic to slow Nic metabolism, it does stand to reason that this effect was more likely due to the weak agonist-like CNS effects of β -Nic at $\alpha 4\beta 2$ nAChRs, as a 10-min pretreatment and the session duration was likely insufficient for β -Nic's effects on Nic metabolism to manifest. To address this issue, a 60-min pretreatment interval was employed for combination testing. Under the 60-min pretreatment interval, rats of both sexes were better able to discriminate Nic when it was co-administered with β -Nic, especially at higher β -Nic doses.

These findings imply that the interoceptive effects of Nic were prolonged by the ability of β -Nic to slow Nic metabolism by inhibiting CYP450 liver function (Kramlinger et al., 2012). During the nicotine alone condition at the 60-min pretreatment interval, female rats were better able to discriminate the training dose of Nic (0.2 mg/kg) compared to males (Figure 4 – top two panels). Response rates again showed a rate-dependent effect akin to substitution testing (Figure 5), wherein females had significantly lower baseline response rates than males that were dose-dependently increased by Nic.

4.4. Acquisition of Nornic and β -Nic Drug Discrimination in Nic naïve rats. The inability of β -Nic to serve as a discriminative stimulus in naïve rats (Figure 6) is somewhat consistent with the Nic substitution data (Figure 2). Both male and female rats were unable to discriminate injections of 2 and 5 mg/kg of β -Nic vs Sal, whereas they were able to discriminate the higher dose of Nornic with some (%33) achieving reliable discrimination (i.e., >80%). These data call into question the reliability of the female β -Nic data showing that it can weakly substitute for Nic. This discrepancy may be due to differences in drug discrimination training history, wherein when trained with Nic female rats may be more sensitive to the interoceptive stimulus effects of β -Nic.

4.5. Scientific & Regulatory Implications. Teenagers are more prone to develop Nic dependence than adults (Adriani et al., 2002; Breslau and Peterson, 1996; DiFranza et al., 2002; Krishnan et al., 2022; Patton et al., 1998). Slower Nic metabolism is a risk factor for Nic dependence in naïve adolescents initiating cigarette smoking (Karp et al., 2006; for a review see Ray et al., 2009). Because slower metabolism results in prolonged systemic Nic exposure, it stands to reason that β -Nic could facilitate development of ENDS dependence in adolescents. Studies in humans are needed to examine this issue. If apparent, the FDA CTP could add β -Nic to its list of HPHCs and require manufacturers to report β -Nic levels in ENDS liquids. If necessary, a β -Nic product standard could be set to prevent increasing β -Nic levels in ENDS.

The present findings show that β -Nic may slow Nic metabolism sufficiently to increase the potency of Nic (e.g., by lowering its discrimination threshold). Prior human laboratory work by Perkins, Sofuoglu, and colleagues has examined the reinforcement and discrimination thresholds for Nic (i.e., see reviews by Perkins et al., 2022, Sofuoglu & LeSage, 2012) and the general conclusions were that greater sensitivity to the interoceptive stimulus effects of Nic is associated with greater abuse liability of Nic (e.g., Perkins et al., 2018). They contend that the discrimination threshold is a stronger metric than that of the reinforcement threshold as the former is necessary for the latter to be observed (Perkins, 2022) and that it should be the target for tobacco control (Sofuoglu & LeSage, 2012). If the present findings are applicable to humans, it suggests that β -Nic should lower the Nic discrimination threshold and thus should result in greater abuse liability of ENDS products.

Figure Captions

Figure 1. **(A)** Inhibition curve showing total nicotine radioligand binding (DPM) across a range of Nicotine, β -Nic and Nornic doses (-5 = 10 μ M, -6 = 1 μ M, etc.). **(B)** Concentration-effect curve for stimulation of $^{86}\text{Rb}^+$ efflux across a range of Nicotine, β -Nic and Nornic doses in Mols (M) as a percentage of 100 μ M of nicotine. (-4 = 100 μ M, -5 = 10 μ M, etc.). **(C)** Concentration-effect curves for β -Nic and Nornic showing the percent inhibition of 100 μ M nicotine on $^{86}\text{Rb}^+$ efflux.

Figure 2. Percent nicotine lever responding and response rate (r/s) during the 2-min nicotine substation probes in male and female rats administered Nic, Nornic or β -Nic 10-min prior to the start of the session. † - indicates a main effect of sex. & - indicates interaction of sex x dose and at what dose post-hoc tests revealed differences. * and # - indicates a significant Sidak post-hoc difference from saline in females and males, respectively.

Figure 3. Percent nicotine lever responding during the 2-min test session as a function of Nic dose (note log axis) during Nic alone and Nic + 0.05/0.25/1.0 β -Nic under a 10-minute pretreatment schedule. Note that the Nic alone cure is the same in each panel within each sex to facilitate comparisons to combination treatments. # denotes a significant main effect of β -Nic. & denotes a significant interaction with β -Nic. * denotes a significant difference from Nic alone during post-hoc tests.

Figure 4. Percent nicotine lever responding during the 2-min test session as a function of Nic dose (note log axis) during Nic alone and Nic + 0.05/0.25/1.0 β -Nic under a 60-minute pretreatment schedule. # denotes a significant main effect of, or an interaction with β -Nic. * denotes a significant difference from Nic alone during post-hoc tests. † - indicates a significant difference between sexes during post-hoc tests of the Nic alone condition.

Figure 5. Response rates (responses/second; r/s) during the 2-min test session across β -Nic doses under the 10- and 60-min pretreatment intervals in male and female rats. *&# each denote a significant difference from Sal from Nic alone and Nic + 0.025/0.05/1.0 β -Nic combination doses, respectively, during post-hoc tests. † - indicates a significant difference between sexes across the bracketed Nic doses collapsing across β -Nic dose.

Figure 6. The **left panel** shows the percent drug appropriate lever responding across training sessions with 2 and 5 mg/kg training dose of β -Nic and Nornic and the **right panel** shows the individual averages of the last three sessions in each Nornic training session in male and female rats. * denotes significant difference between Nornic and β -Nic during the final 3 training sessions.

Figure 1.

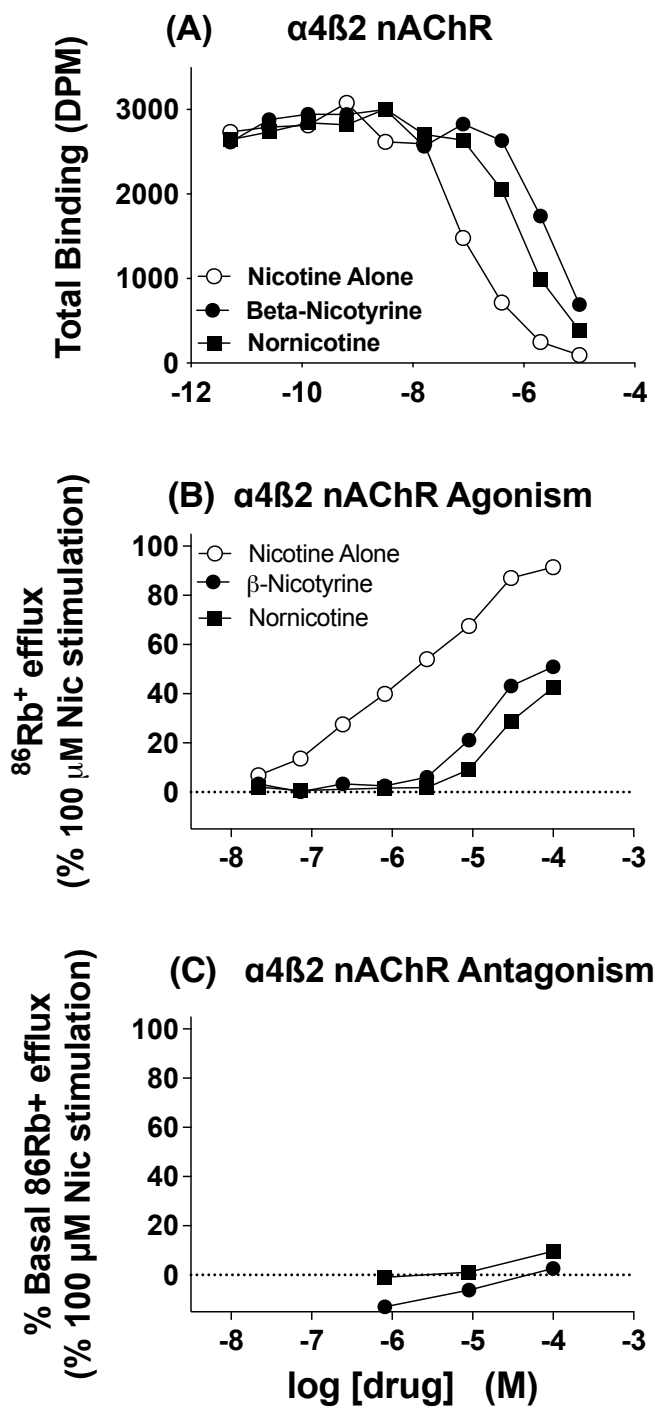


Figure 2.

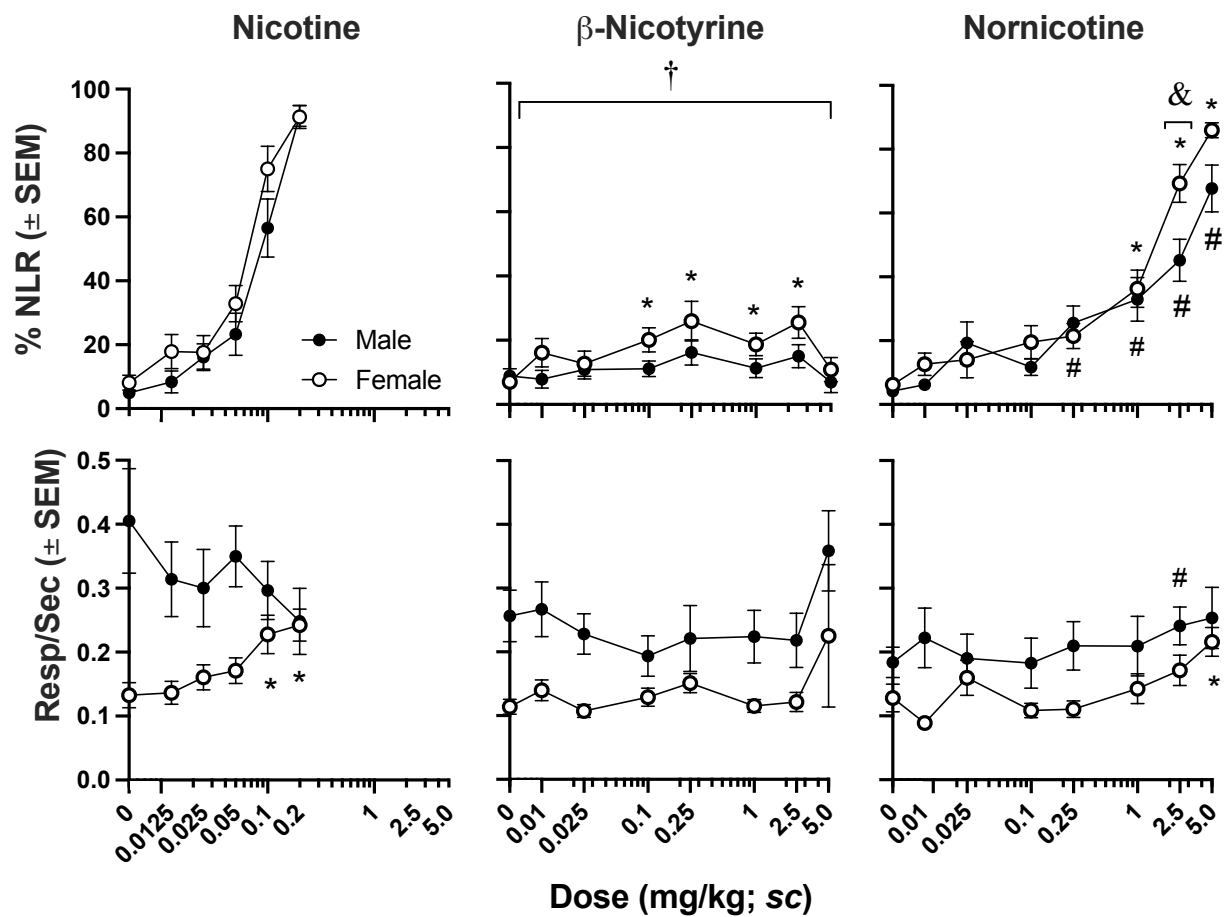


Figure 3.

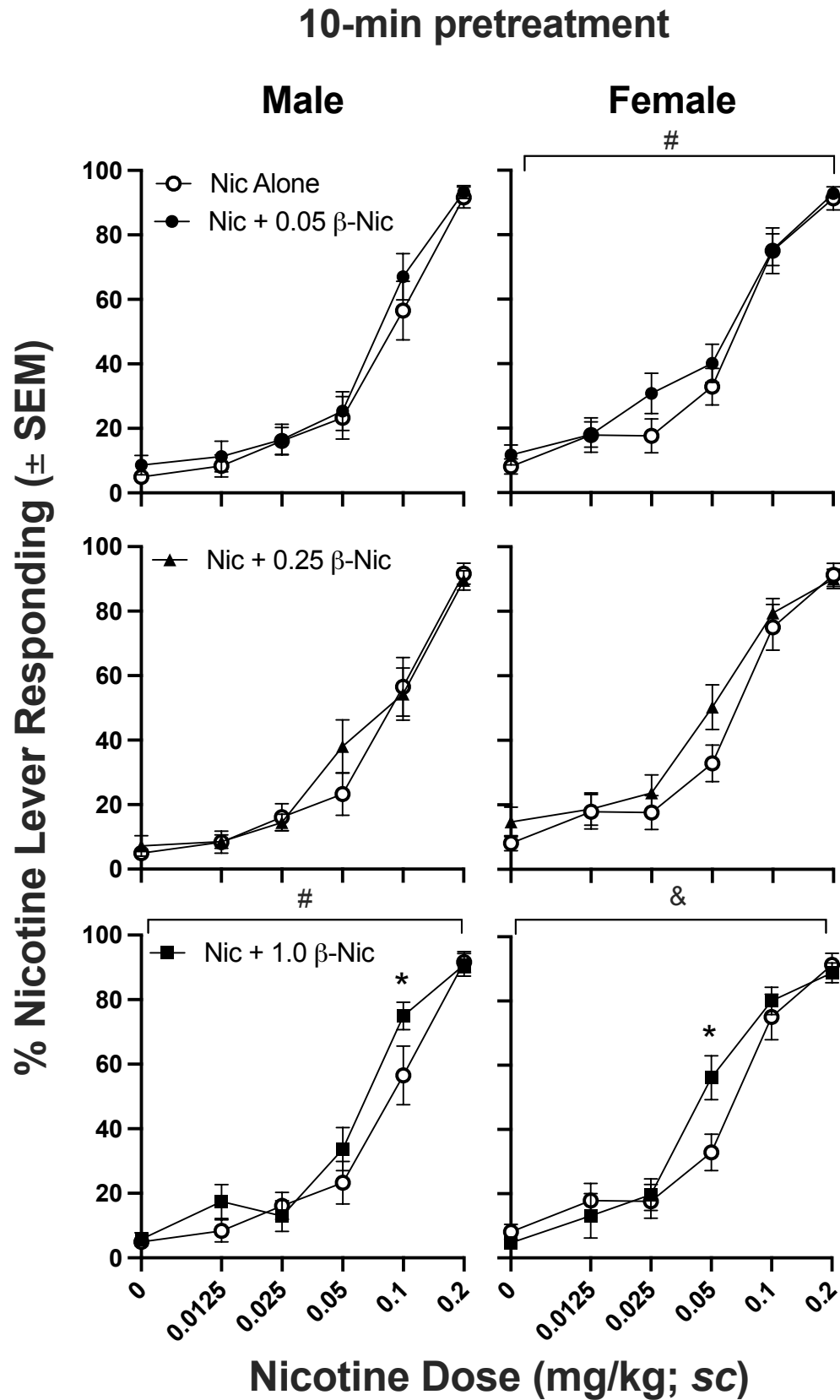


Figure 4.

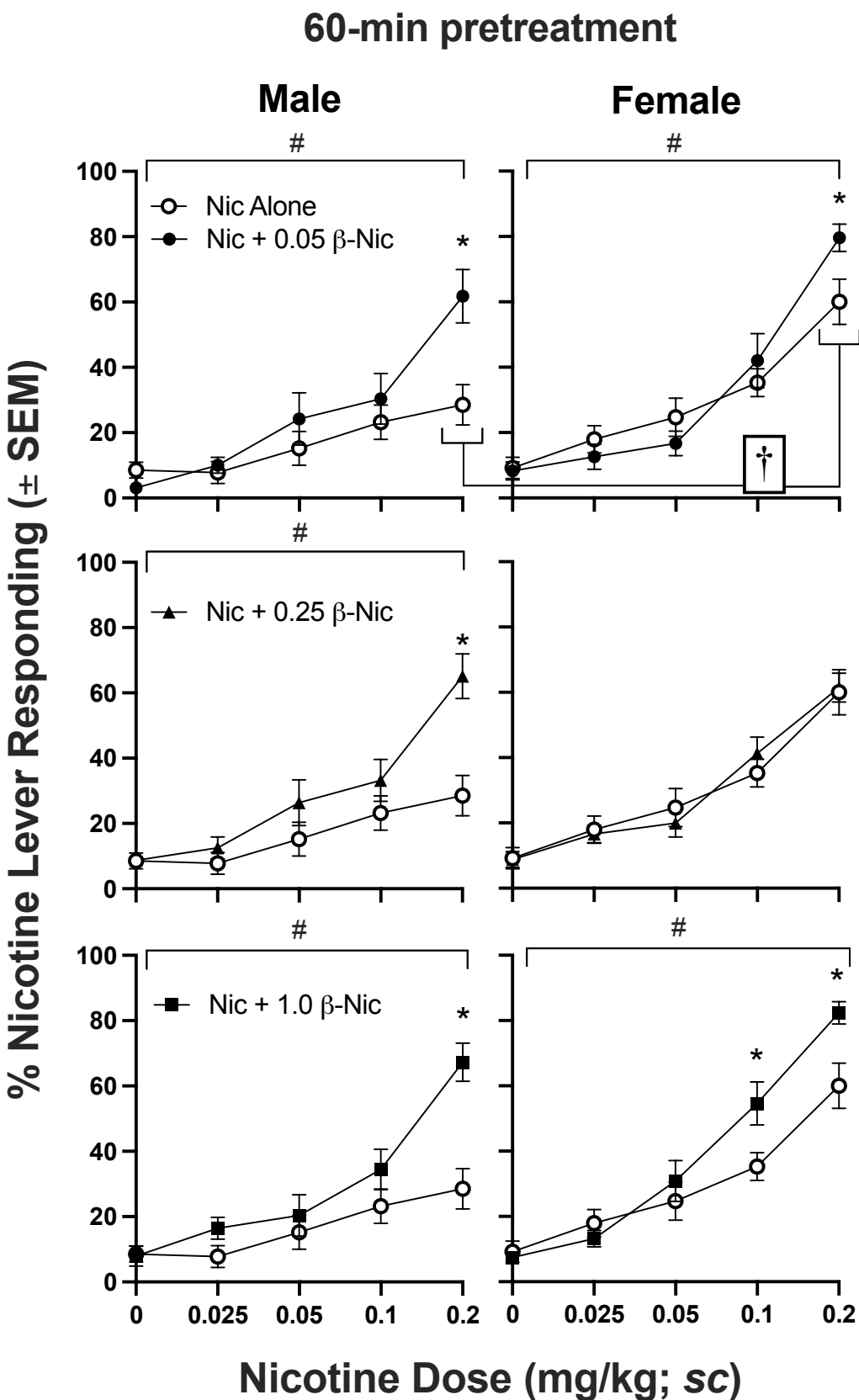


Figure 5.

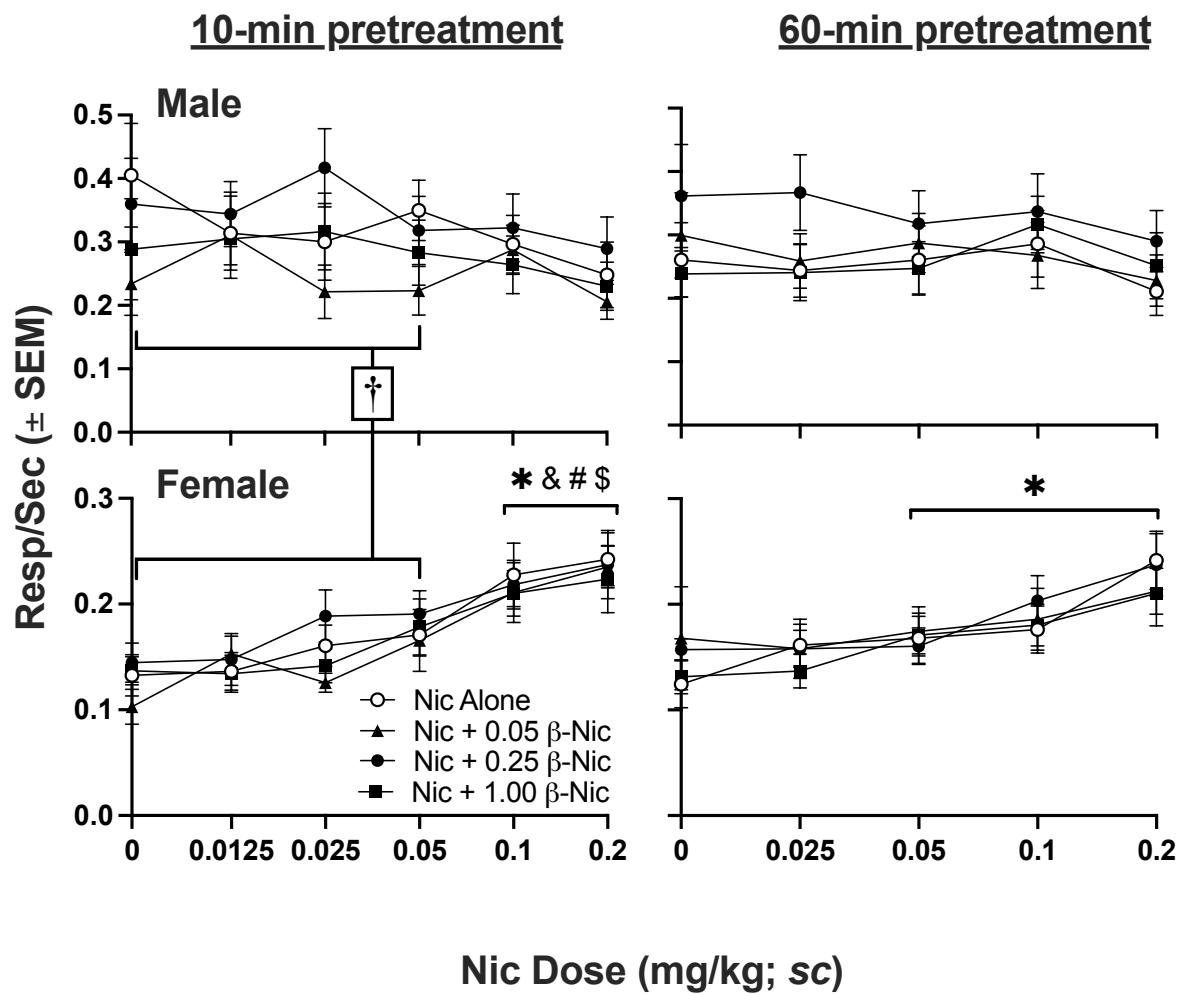
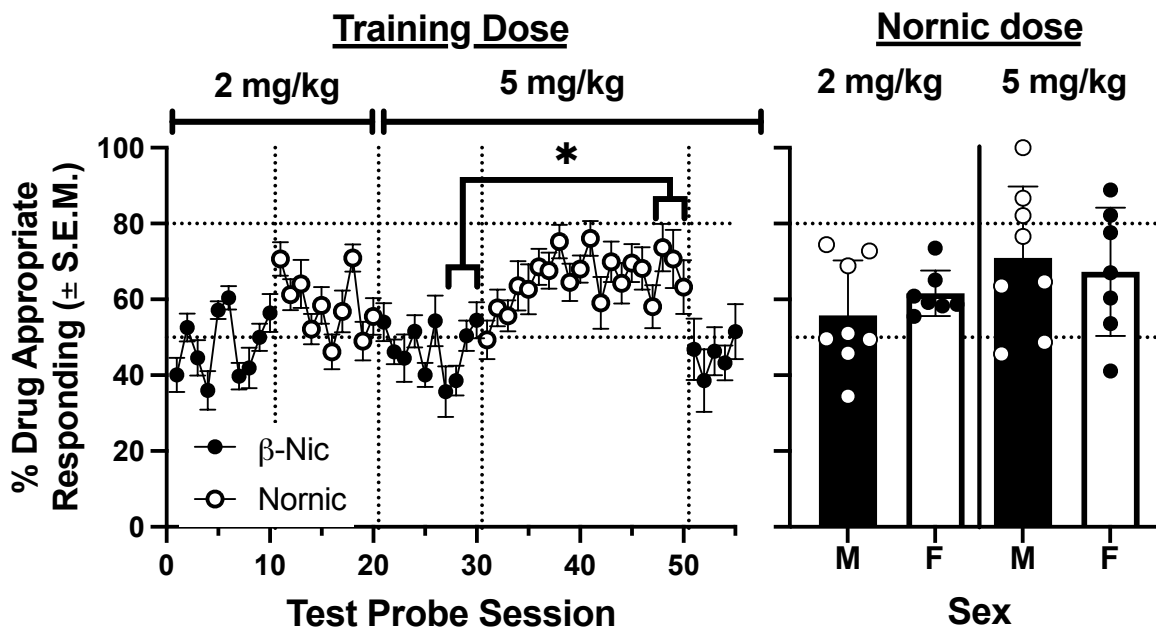


Figure 6.



Contributions

JRS conceptualized the study and experimental design. MGL, JRS, and AHR finalized the experimental protocols. SW and PM were responsible for daily conduct of the study and data collection. JRS supervised conduct of the study and analyzed the data. JRS drafted the manuscript and MGL and AH provided editorial comments and suggestions. All authors reviewed drafts of the manuscript and approved the final version.

Conflict of Interest

No conflict declared.

Role of Funding Source

This study was supported by NIDA grant R01-DA053608 (Smethells JR, PI) and a Career Development Award from Hennepin Healthcare Research Institute (JRS). These funding institutions had no role in study design, data collection and analysis, or the decision to submit the manuscript for publication.

Acknowledgements

We would like to thank Sam Howard and Jennifer Vigliaturo for their excellent technical assistance. Ki determinations, receptor binding profiles, agonist and antagonist functional data was generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # HHSN-271-2018-00023-C (NIMH PDSP). The NIMH PDSP is Directed by Bryan L. Roth at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscoll at NIMH, Bethesda MD, USA. For experimental details please refer to the PDSP web site <https://pdsp.unc.edu/ims/investigator/web/>.

References

- Abramovitz, A., McQueen, A., Martinez, R.E., Williams, B.J., Sumner, W., 2015. Electronic cigarettes: The nicotine hypothesis. *Med. Hypotheses* 85, 305–310. <https://doi.org/10.1016/j.mehy.2015.06.002>
- Adriani, W., Macrí, S., Pacifici, R., Laviola, G., 2002. Peculiar vulnerability to nicotine oral self-administration in mice during early adolescence. *Neuropsychopharmacology* 27, 212–224. doi:10.1016/S0893-133X(02)00295-6
- Ahsan, H. M., Pena, J. B. I., Botanas, C. J., Kim, H. J., Yu, G. Y., Cheong, J. H., 2014. Conditioned place preference and self-administration induced by nicotine in adolescent and adult rats. *Biomol Ther*, 22, 460-466.
- Alkhalif, Y., Shelton, K. L., 2023. Assessment of aubse-related discriminative stimulus effects of nicotine aerosol in rodents. *J. Pharmacol Exp Therapeutics* 385, 171-179.
- Arrazola, R.A., Singh, T., Corey, C.G., Husten, C.G., Neff, L.J., Apelberg, B.J., Bunnell, R.E., Choiniere, C.J., King, B.A., Cox, S., McAfee, T., Caraballo, R.S., Centers for Disease Control and Prevention (CDC), 2015. Tobacco use among middle and high school students - United States, 2011-2014. *MMWR. Morb. Mortal. Wkly. Rep.* 64, 381–5.
- Ator, N.A., Griffiths, R.R., 2003. Principles of drug abuse liability assessment in laboratory animals. *Drug Alcohol Depend.* 70, S55–S72. [https://doi.org/10.1016/S0376-8716\(03\)00099-1](https://doi.org/10.1016/S0376-8716(03)00099-1)
- Bardo, M.T., Green, T.A., Crooks, P.A., Dwoskin, L.P., 1999. Nicotine is self-administered intravenously by rats. *Psychopharmacology (Berl)*. 146, 290–296. <https://doi.org/10.1007/s002130051119>
- Benowitz, N., 2010. Nicotine Addiction. *N. Engl. J. Med.* 362, 2295–2303. <https://doi.org/10.1056/NEJMra0809890>. Nicotine
- Benowitz, N.L., Dains, K.M., Hall, S.M., Stewart, S., Wilson, M., Dempsey, D., Jacob, P., 2012. Smoking behavior and exposure to tobacco toxicants during 6 months of smoking progressively reduced nicotine content cigarettes. *Cancer Epidemiol. Biomarkers Prev.* 21, 761–769. <https://doi.org/10.1158/1055-9965.EPI-11-0644>
- Benowitz, N.L., Donny, E.C., Hatsukami, D.K., 2016. Reduced nicotine content cigarettes, e-cigarettes and the cigarette end game. *Addiction* 6–7. <https://doi.org/10.1111/add.13534>
- Benowitz, N. L., & Hatsukami, D. (1998). Gender differences in the pharmacology of nicotine addiction. *Addiction biology*, 3(4), 383-404.
- Breslau, N., Peterson, E.L., 1996. Smoking cessation in young adults: Age at initiation of cigarette smoking and other suspected influences. *Am. J. Public Health* 86, 214–220. <https://doi.org/10.2105/AJPH.86.2.214>
- Bunney, P.E., Smethells, J.R., LeSage, M.G. 2023. Substitutability of nicotine and sucrose in rats: A behavioral economic analysis. *Pharmacol Biochem Behav*, 232, 173635.
- Caggiula, A.R., Donny, E.C., Palmatier, M.I., Liu, X., Chaudhri, N., Sved, A.F., 2009. The role of nicotine in smoking: A dual-reinforcement model. *Nebraska Symp. Motiv.* 55, 91–109. https://doi.org/10.1007/978-0-387-78748-0_6
- Caine, S.B., Collins, G.T., Thomsen, M., Wright, C., Lanier, R.K., Mello, N.K., 2014. Nicotine-like behavioral effects of the minor tobacco alkaloids nornicotine, anabasine, and anatabine in male rodents. *Exp. Clin. Psychopharmacol.* 22, 9–22. <https://doi.org/10.1037/a0035749>

- Carroll, M.E., Lynch, W.J., 2016. How to study sex differences in addiction using animal models. *Addict. Biol.* 21, 1007–1029. doi:10.1111/adb.12400
- Carroll, M.E., Lynch, W.J., Roth, M.E., Morgan, A.D., Cosgrove, K.P., 2004. Sex and estrogen influence drug abuse. *Trends Pharmacol. Sci.* 25, 273–279. doi:10.1016/j.tips.2004.03.011
- Carroll, M.E., Meisch, R.A., 1978. Etonitazene as a reinforcer: Oral intake of etonitazene by rhesus monkeys. *Psychopharmacology (Berl)*. 59, 225–229. doi:10.1007/BF00426626
- Carroll, M.E., Smethells, J.R., 2016. Sex differences in behavioral dyscontrol: Role in drug addiction and novel treatments. *Front. Psychiatry* 6, 1–20. doi:10.3389/fpsy.2015.00175
- Cengelli, S., O’Loughlin, J., Lauzon, B., Cornuz, J., 2012. A systematic review of longitudinal population-based studies on the predictors of smoking cessation in adolescent and young adult smokers. *Tob. Control* 21, 355–362. doi:10.1136/tc.2011.044149
- Chaudhri, N., Caggiula, A.R., Donny, E.C., Booth, S., Gharib, M.A., Craven, L.A., Allen, S.S., Sved, A.F., Perkins, K.A., 2005. Sex differences in the contribution of nicotine and nonpharmacological stimuli to nicotine self-administration in rats. *Psychopharmacology (Berl)*. 180, 258–266. doi:10.1007/s00213-005-2152-3
- Chellian, R., Behnood-Rod, A., Wilson, R., Kamble, S.H., Sharma, A., McCurdy, C.R., Bruijnzeel, A.W., 2020. Adolescent nicotine and tobacco smoke exposure enhances nicotine self-administration in female rats. *Neuropharmacology* 176, 108243. doi:10.1016/j.neuropharm.2020.108243
- Clayton, P., Lu, A., Bishop, L., 2009. The pyrolysis of (-)-(S)-nicotine: Racemization and decomposition. *Chirality* 18, NA-NA. <https://doi.org/10.1002/chir.20760>
- Cohen, A., Koob, G.F., George, O., 2012. Robust escalation of nicotine intake with extended access to nicotine self-administration and intermittent periods of abstinence. *Neuropsychopharmacology* 37, 2153–2160. <https://doi.org/10.1038/npp.2012.67>
- Cohen, J., 1992. A power primer. *Psychol. Bull.* 112, 155–159. <https://doi.org/10.1037/0033-2909.112.1.155>
- Denton, T.T., Zhang, X., Cashman, J.R., 2004. Nicotine-related alkaloids and metabolites as inhibitors of human cytochrome P-450 2A6. *Biochem. Pharmacol.* 67, 751–756. <https://doi.org/10.1016/j.bcp.2003.10.022>
- DiFranza, J.R., Savageau, J.A., Rigotti, N.A., Fletcher, K., Ockene, J.K., McNeill, A.D., Coleman, M., Wood, C., 2002. Development of symptoms of tobacco dependence in youths: 30 month follow up data from the DANDY study. *Tob. Control* 11, 228–35. doi:10.1136/tc.11.3.228
- Donny, E.C., Bigelow, G.E., Walsh, S.L., 2006. Comparing the physiological and subjective effects of self-administered vs yoked cocaine in humans. *Psychopharmacology (Berl)*. 186, 544–552. doi:10.1007/s00213-006-0312-8
- Donny, E.C., Caggiula, A.R., Rowell, P.P., Gharib, M.A., Maldovan, V., Booth, S., Mielke, M.M., Hoffman, A., McCallum, S., 2000. Nicotine self-administration in rats: Estrous cycle effects, sex differences and nicotinic receptor binding. *Psychopharmacology (Berl)*. 151, 392–405. doi:10.1007/s002130000497
- Donny, E.C., Denlinger, R.L., Tidey, J.W., Koopmeiners, J.S., Benowitz, N.L., Vandrey, R.G., Al’Absi, M., Carmella, S.G., Cinciripini, P.M., Dermody, S.S., Drobos, D.J., Hecht, S.S., Jensen, J., Lane, T., Le, C.T., McClernon, F.J., Montoya, I.D., Murphy, S.E., Robinson, J.D., Stitzer, M.L., Strasser, A.A., Tindle, H., Hatsukami, D.K., 2015. Randomized Trial of Reduced-Nicotine Standards for Cigarettes. *N. Engl. J. Med.* 373, 1340–1349. doi:10.1056/NEJMsa1502403

- Donny, E.C., Houtsmuller, E., Stitzer, M.L., 2007. Smoking in the absence of nicotine: Behavioral, subjective and physiological effects over 11 days. *Addiction* 102, 324–334. doi:10.1111/j.1360-0443.2006.01670.x
- Donny, E.C., Taylor, T.G., LeSage, M.G., Levin, Me., Buffalari, D.M., Joel, D., Sved, A.F., 2012. Impact of tobacco regulation on animal research: new perspectives and opportunities. *Nicotine & Tobacco Research* 14, 1319–1338. <https://doi.org/10.1093/ntr/nts162>
- Evans, S. M. 2007. The role of estradiol and progesterone in modulating the subjective effects of stimulants in humans. *Exp Clin Psychopharm* 15, 418-426.
- Faul, F., Erdfelder, E., Lang, A.-G., Buchner, A., 2007. G*Power: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods* 39, 175–191. doi:10.3758/BF03193146
- Flora, J.W., Wilkinson, C.T., Wilkinson, J.W., Lipowicz, P.J., Skapars, J.A., Anderson, A., Miller, J.H., 2017. Method for the Determination of Carbonyl Compounds in E-Cigarette Aerosols. *J. Chromatogr. Sci.* 55, 142–148. <https://doi.org/10.1093/chromsci/bmw157>
- Flores, R.J., Uribe, K.P., Swalve, N., O'Dell, L.E., 2019. Sex differences in nicotine intravenous self-administration: A meta-analytic review. *Physiol. Behav.* 203, 42–50. doi:10.1016/j.physbeh.2017.11.017
- Food and Drug Administration [FDA], 2016. Deeming Tobacco Products To Be Subject to the Federal Food, Drug, and Cosmetic Act, as Amended by the Family Smoking Prevention and Tobacco Control Act; Restrictions on the Sale and Distribution of Tobacco Products and Required Warning Statements for Tobacco Products. Final rule. Federal Registrar: 2016;90:28973-29106.
- FDA, 2018. Guidance for Industry Listing of Ingredients in Tobacco Products (Docket #: FDA-2009-D-0524-0002).
- Glasser, A.M., Collins, L., Pearson, J.L., Abudayyeh, H., Niaura, R.S., Abrams, D.B., Villanti, A.C., 2017. Overview of Electronic Nicotine Delivery Systems: A Systematic Review. *Am. J. Prev. Med.* 52, e33–e66. <https://doi.org/10.1016/j.amepre.2016.10.036>
- Gasior, M., Shoaib, M., Yasar, S., Jaszyna, M., Goldberg, S.R., 1999. Acquisition of nicotine discrimination and discriminative stimulus effects of nicotine in rats chronically exposed to caffeine. *J. Pharmacol. Exp. Ther.* 288, 1053–1073.
- Goldberg, S.R., Risner, M.E., Stolerman, I.P., Reavill, C., Garcha, H.S., 1989. Nicotine and some related compounds: effects on schedule-controlled behaviour and discriminative properties in rats. *Psychopharmacology (Berl)*. 97, 295–302. <https://doi.org/10.1007/BF00439441>
- Gourlay, S.G., Benowitz, N.L., 1997. Arteriovenous differences in plasma concentration of nicotine and catecholamines and related cardiovascular effects after smoking, nicotine nasal spray, and intravenous nicotine. *Clin. Pharmacol. Ther.* 62, 453–463. [https://doi.org/10.1016/S0009-9236\(97\)90124-7](https://doi.org/10.1016/S0009-9236(97)90124-7)
- Govind, A. P., Vezina, P., Green, W. N., 2009. Nicotine-induced upregulation of nicotine receptors: Underlying mechanisms and relevance to nicotine addiction. 78, 756-765.
- Grebenstein, P.E., Burroughs, D., Roiko, S.A., Pentel, P.R., LeSage, M.G., 2015. Predictors of the nicotine reinforcement threshold, compensation, and elasticity of demand in a rodent model of nicotine reduction policy. *Drug Alcohol Depend.* 151, 181–193. <https://doi.org/10.1016/j.drugalcdep.2015.03.030>
- Harris, A.C., Mattson, C., Shelley, D., Lesage, M.G., 2014. Restraint stress attenuates nicotine's locomotor stimulant but not discriminative stimulus effects in rats. *Pharmacol. Biochem.*

Behav. 124, 92–100. doi:10.1016/j.pbb.2014.05.012

Harris, A.C., Muelken, P., Smethells, J.R., Yershova, K., Stepanov, I., Olson, T.T., Kellar, K.J., LeSage, M.G., 2018. Effects of nicotine-containing and “nicotine-free” e-cigarette refill liquids on intracranial self-stimulation in rats. *Drug Alcohol Depend.* 185, 1–9. doi:10.1016/j.drugalcdep.2017.11.032

Harris, A.C., Muelken, P., Swain, Y., Palumbo, M., Jain, V., Goniewicz, M.L., Stepanov, I., LeSage, M.G., 2019. Non-nicotine constituents in e-cigarette aerosol extract attenuate nicotine’s aversive effects in adolescent rats. *Drug Alcohol Depend.* 203, 51–60. doi:10.1016/j.drugalcdep.2019.05.023

Harris, A.C., Stepanov, I., Pentel, P.R., LeSage, M.G., 2012. Delivery of nicotine in an extract of a smokeless tobacco product reduces its reinforcement-attenuating and discriminative stimulus effects in rats. *Psychopharmacology (Berl.)* 220, 565–576. doi:10.1007/s00213-011-2514-y

Hatsukami, D.K., Jensen, J.A., Carroll, D.M., Luo, X., Strayer, L.G., Cao, Q., Hecht, S.S., Murphy, S.E., Carmella, S.G., Denlinger-Apte, R.L., Colby, S., Strasser, A.A., McClernon, F.J., Tidey, J., Benowitz, N.L., Donny, E.C., 2024. Reduced nicotine in cigarettes in a marketplace with alternative nicotine systems: randomized clinical trial. *Lancet Reg. Heal. - Am.* 35, 100796. <https://doi.org/10.1016/j.lana.2024.100796>

Donny, E.C., Taylor, T.G., LeSage, M.G., Levin, Me., Buffalari, D.M., Joel, D., Sved, A.F., 2012. Impact of tobacco regulation on animal research: new perspectives and opportunities. *Nicotine & Tobacco Research* 14, 1319–1338. <https://doi.org/10.1093/ntr/nts162>

Hoffman, A.C., Evans, S.E., 2013. Abuse Potential of Non-Nicotine Tobacco Smoke Components: Acetaldehyde, Nicotine, Cotinine, and Anabasine. *Nicotine Tob. Res.* 15, 622–632. <https://doi.org/10.1093/ntr/nts192>

Jacob, P., Yu, L., Shulgin, A.T., Benowitz, N.L., 1999. Minor tobacco alkaloids as biomarkers for tobacco use: Comparison of users of cigarettes, smokeless tobacco, cigars, and pipes. *Am. J. Public Health* 89, 731–736. <https://doi.org/10.2105/AJPH.89.5.731>

Jensen, K.P., DeVito, E.E., Sofuoglu, M., 2016. How Intravenous Nicotine Administration in Smokers Can Inform Tobacco Regulatory Science. *Tob. Regul. Sci.* 2, 452–463. <https://doi.org/10.18001/TRS.2.4.14>

Johnston, L.D., Miech, R.A., O’Malley, P.M., Bachman, J.G., Schulenberg, J.E., Patrick, M.E., 2019. Monitoring the Future national survey results on drug use 1975–2018: Overview, key findings on adolescent drug use.

Karp, I., O’Loughlin, J., Hanley, J., Tyndale, R.F., Paradis, G., 2006. Risk factors for tobacco dependence in adolescent smokers. *Tob. Control* 15, 199–204. <https://doi.org/10.1136/tc.2005.014118>

Keenan, K., 1999. Diet, caloric restriction, and the rodent bioassay. *Toxicol. Sci.* 52, 24–34. https://doi.org/10.1093/toxsci/52.suppl_1.24

Khuder, S., Dayal, H., Mutgi, A., 1999. Age at smoking onset and its effect on smoking cessation.; - Addictive behaviors. - *Addict. Behav.* 24, 673–677.

Koob, G.F., 2000. Neurobiology of addiction: Toward the development of new therapies. *Ann. N. Y. Acad. Sci.* 909, 170–185. <https://doi.org/10.1111/j.1749-6632.2000.tb06682.x>

Kornetsky, C., Esposito, R.U., 1979. Euphorogenic drugs: effects on the reward pathways of the brain. *Fed. Proc.* 38, 2473–6.

- Kramlinger, V.M., von Weyarn, L.B., Murphy, S.E., 2012. Inhibition and inactivation of cytochrome P450 2A6 and cytochrome P450 2A13 by menthofuran, β -nicotyrine and menthol. *Chem. Biol. Interact.* 197, 87–92. doi:10.1016/j.cbi.2012.03.009
- Krishnan, N., Berg, C.J., Elmi, A.F., Klemperer, E.M., Sherman, S.E., Abrams, L.C., 2022. Trajectories of ENDS and cigarette use among dual users : analysis of waves 1 to 5 of the PATH Study 1–7. doi:10.1136/tobaccocontrol-2022-057405
- Leao, R.M., Cruz, F.C., Vendruscolo, L.F., de Guglielmo, G., Logrip, M.L., Planeta, C.S., Hope, B.T., Koob, G.F., George, O., 2015. Chronic Nicotine Activates Stress/Reward-Related Brain Regions and Facilitates the Transition to Compulsive Alcohol Drinking. *J. Neurosci.* 35, 6241–6253. <https://doi.org/10.1523/JNEUROSCI.3302-14.2015>
- LeSage, Mark G, Burroughs, D., Muelken, P., Harris, A.C., 2016. Self-administration of Smokeless Tobacco Products in Rats 2, 329–342. doi:10.18001/TRS.2.4.5
- LeSage, M.G., Keyler, D.E., Collins, G., Pentel, P.R., 2003. Effects of continuous nicotine infusion on nicotine self-administration in rats: Relationship between continuously infused and self-administered nicotine doses and serum concentrations. *Psychopharmacology (Berl)*. 170, 278–286. doi:10.1007/s00213-003-1539-2
- LeSage, M.G., Keyler, D.E., Hieda, Y., Collins, G., Burroughs, D., Le, C., Pentel, P.R., 2006. Effects of a nicotine conjugate vaccine on the acquisition and maintenance of nicotine self-administration in rats. *Psychopharmacology (Berl)*. 184, 409–416. doi:10.1007/s00213-005-0027-2
- LeSage, M.G., Keyler, D.E., Shoeman, D., Raphael, D., Collins, G., Pentel, P.R., 2002. Continuous nicotine infusion reduces nicotine self-administration in rats with 23-h/day access to nicotine. *Pharmacol. Biochem. Behav.* 72, 279–289. doi:10.1016/S0091-3057(01)00775-4
- LeSage, M.G., Shelley, D., Pravetoni, M., Pentel, P.R., 2012. Enhanced attenuation of nicotine discrimination in rats by combining nicotine-specific antibodies with a nicotinic receptor antagonist. *Pharmacol. Biochem. Behav.* 102, 157–162. doi:10.1016/j.pbb.2012.03.026
- LeSage, M.G., Shelley, D., Ross, J.T., Carroll, F.I., Corrigan, W.A., 2009. Effects of the nicotinic receptor partial agonists varenicline and cytisine on the discriminative stimulus effects of nicotine in rats. *Pharmacol. Biochem. Behav.* 91, 461–467. doi:10.1016/j.pbb.2008.08.024
- LeSage, M.G., Smethells, J.R., Harris, A.C., 2018. Status and future directions of preclinical behavioral pharmacology in tobacco regulatory science. *Behav. Anal. Res. Pract.* 18, 252–274. doi:<http://dx.doi.org.ezproxy1.lib.asu.edu/10.1037/bar0000113>
- LeSage, M. G., Staley, M., Muelken, P., Smethells, J.R., Stepanov, I., Vogel, R.I., Pentel, P.R., Harris, A.C., 2016. Abuse liability assessment of an e-cigarette refill liquid using intracranial self-stimulation and self-administration models in rats. *Drug Alcohol Depend.* 168, 76–88. doi:10.1016/j.drugalcdep.2016.08.628
- Leslie, F. M., 2020. Unique long-term effects of nicotine on adolescent brain. *Pharm Bio Behav.* 197, 173010. doi: 10.1016/j.pbb.2020.173010.
- Levin, E.D., Rezvani, A.H., Montoya, D., Rose, J.E., Scott Swartzwelder, H., 2003. Adolescent-onset nicotine self-administration modeled in female rats. *Psychopharmacology (Berl)*. 169, 141–149. doi:10.1007/s00213-003-1486-y
- Li, S., Zou, S., Coen, K., Funk, D., Shram, M.J., Lê, A.D., 2014. Sex differences in yohimbine-induced increases in the reinforcing efficacy of nicotine in adolescent rats. *Addict. Biol.* 19, 156–164. doi:10.1111/j.1369-1600.2012.00473.x
- Liu, X., Zang, L., Van Der Schyf, C.J., Igarashi, K., Castagnoli, K., Castagnoli, N., 1999. Studies

on the pyrrolinone metabolites derived from the tobacco alkaloid 1-methyl-2-(3-pyridinyl)pyrrole (β -nicotyrine). *Chem. Res. Toxicol.* 12, 508–512.
<https://doi.org/10.1021/tx990019j>

- Lynch, W.J., 2009. Sex and ovarian hormones influence vulnerability and motivation for nicotine during adolescence in rats. *Pharmacol. Biochem. Behav.* 94, 43–50.
doi:10.1016/j.pbb.2009.07.004
- Lynch, W.J., 2006. Sex differences in vulnerability to drug self-administration. *Exp. Clin. Psychopharmacol.* 14, 34–41. doi:10.1037/1064-1297.14.1.34
- Lynch, W.J., Roth, M.E., Carroll, M.E., 2002. Biological basis of sex differences in drug abuse: Preclinical and clinical studies. *Psychopharmacology (Berl)*. 164, 121–137.
doi:10.1007/s00213-002-1183-2
- Martinez, R.E., Dhawan, S., Sumner, W., Williams, B.J., 2015. On-line chemical composition analysis of refillable electronic cigarette aerosol- measurement of nicotine and nicotyrine. *Nicotine Tob. Res.* 17, 1263–1269. doi:10.1093/ntr/ntu334
- Marusich, J.A., Darna, M., Wilson, A.G., Denehy, E.D., Ebben, A., Deaciuc, A.G., Dwoskin, L.P., Bardo, M.T., Lefever, T.W., Wiley, J.L., Reissig, C.J., Jackson, K.J., 2017. Tobacco's minor alkaloids: Effects on place conditioning and nucleus accumbens dopamine release in adult and adolescent rats. *Eur. J. Pharmacol.* 814, 196–206. doi:10.1016/j.ejphar.2017.08.029
- Marusich, J. A. & Palmatier, M. I. Development of a nicotine aerosol self-administration model in rats and the effects of e-liquid flavors. *Behav Pharmacol* 34, 141–153 (2023).
- Meich, R. A., Johnston, L. D., O'Malley, P.M., Bachman, J.G., Schulenberg, J. E. & Patrick, M.E., 2022. Monitoring the future national survey results on drug use, 1975-2021: Volume I, Secondary school students.
- National Academies of Sciences Engineering and Medicine, 2018. Public Health Consequences of E-Cigarettes. National Academies Press, Washington, D.C. doi:10.17226/24952
- Negus, S.S., Miller, L.L., 2014. Intracranial Self-Stimulation to Evaluate Abuse Potential of Drugs. *Pharmacol. Rev.* 66, 869–917. doi:10.1124/pr.112.007419
- NIDA, 2012. Strategic Plan. Retrieved on 10/02/2019 from: <https://www.drugabuse.gov/about-nida/organization/divisions/division-basic-neuroscience-behavioral-research-dbnbr/strategic-plan>
- Patrick, M. E., Schulenberg, J. E., Miech, R. A., Johnston, L. D., O'Malley, P. M., & Bachman, J. G. (2022). Monitoring the Future Panel Study annual report: National data on substance use among adults ages 19 to 60, 1976-2021. Monitoring the Future Monograph Series. University of Michigan Institute for Social Research: Ann Arbor, MI. doi:10.7826/ISR-UM.06.585140.002.07.0001.2022
- Panlilio, L. V., Goldberg, S.R., 2007. Self-administration of drugs in animals and humans as a model and an investigative tool. *Addiction* 102, 1863–1870. doi:10.1111/j.1360-0443.2007.02011.x
- Patton, G.C., Carlin, J.B., Coffey, C., Wolfe, R., Hibbert, M., Bowes, G., 1998. Depression, anxiety, and smoking initiation: a prospective study over 3 years. *Am. J. Public Health* 88, 1518–22.
- Perkins, K. a, Donny, E., Caggiula, a R., 1999. Sex differences in nicotine effects and self-administration: review of human and animal evidence. *Nicotine Tob. Res.* 1, 301–315.
doi:10.1080/14622299050011431
- Perkins, K.A., Karelitz, J.L., & Kunkle, N. (2018). Evaluation of menthol per se on acute perceptions and behavioral choice of cigarettes differing in nicotine content. *Journal of Psychopharmacology*, 32(3), 324-331. doi: 10.1177/0269881117742660

- Perkins, K.A., 2022. Acute nicotine reinforcement requires ability to discriminate the stimulus effects of nicotine. *Exp. Clin. Psychopharmacol.* 30, 253–268. doi:10.1037/pha0000433
- Perkins, K.A., 2001. Smoking cessation in women - Special considerations. *CNS Drugs* 15, 391–411. doi:10.2165/00023210-200115050-00005
- Perkins, K.A., Fonte, C., Grobe, J.E., 2000. Sex differences in the acute effects of cigarette smoking on the reinforcing value of alcohol. *Behav. Pharmacol.* 11, 63–70. doi:10.1097/00008877-200002000-00007
- Pierce, C., O'Brien, C. P., Kenny, P., Vanderschuren, L. 2012. Rational development of addiction pharmacotherapies: Successes, Failures, and prospects. *Cold Spring Harb Lab Press.* 2, a012880.
- Pogun, S., Yararbas, G., Nesil, T., Kanit, L., 2017. Sex differences in nicotine preference. *J. Neurosci. Res.* 95, 148–162. doi:10.1002/jnr.23858
- Rezvani, A.H., Eddins, D., Slade, S., Hampton, D.S., Christopher, N.C., Petro, A., Horton, K., Johnson, M., Levin, E.D., 2008. Neonatal 6-hydroxydopamine lesions of the frontal cortex in rats: Persisting effects on locomotor activity, learning and nicotine self-administration. *Neuroscience* 154, 885–897. doi:10.1016/j.neuroscience.2008.04.020
- Roth, B.L. 2018. National Institute of Mental Health Psychoactive Drug Screening Program (NIMH PDSP) Assay Protocol Book V. III. Retrieved from: <https://pdsp.unc.edu/ims/investigator/web/docs/UNC-CHProtocolBook.pdf>
- Sanchez, V., Moore, C.F., Brunzell, D.H., Lynch, W.J., 2014. Sex differences in the effect of wheel running on subsequent nicotine-seeking in a rat adolescent-onset self-administration model. *Psychopharmacology (Berl)*. 231, 1753–1762. doi:10.1007/s00213-013-3359-3
- Schuster, C.R., Thompson, T., 1993. Self Administration of and Behavioral Dependence on Drugs, in: *Methods in Behavioral Pharmacology*. pp. 217–243. doi:10.1146/annurev.pa.09.040169.002411
- Shaham, Y., Adamson, L.K., Grocki, S., Corrigall, W.A., 1997. Reinstatement and spontaneous recovery of nicotine seeking in rats. *Psychopharmacology (Berl)*. 130, 396–403. doi:10.1007/s002130050256
- Shao, X.M., Liu, S., Lee, E.S., Fung, D., Pei, H., Liang, J., Mudgway, R., Zhang, J., Feldman, J.L., Zhu, Y., Louie, S., Xie, X.S., 2018. Chronic intermittent nicotine delivery via lung alveolar region-targeted aerosol technology produces circadian pharmacokinetics in rats resembling human smokers. *J. Appl. Physiol.* 125, 1555–1562. doi:10.1152/jappphysiol.00357.2018
- Shigenaga, M.K., Kim, B.H., Caldera-Munoz, P., Cairns, T., Jacob, P., Trevor, A.J., Castagnoli, N., 1989. Liver and lung microsomal metabolism of the tobacco alkaloid .beta.-nicotyrine. *Chem. Res. Toxicol.* 2, 282–287. doi:10.1021/tx00011a003
- Sinden, J. D., Magnen, J. Le., 1981. Parameters of low-dose ethanol intravenous self-administration in the rat. *Pharm. Bio. Behav.*, 16, 181-185.
- Smethells, J.R., Burroughs, D., Saykao, A., Pentel, P.R., Rezvani, A.H., LeSage, M.G., 2021. The reinforcement threshold and elasticity of demand for nicotine in an adolescent rat model of depression. *Drug Alcohol Depend.* 219, 108433. doi:10.1016/j.drugalcdep.2020.108433
- Smethells, J.R., Burroughs, D., Saykao, A., LeSage, M.G., 2023. The relative reinforcing efficacy of nicotine in an adolescent model of attention-deficit hyperactivity disorder. *Frontiers in Psychiatry*, 14, 1154773.

- Smethells, J.R.J.R., Harris, A.C.A.C., Burroughs, D., Hursh, S.R.S.R., LeSage, M.G.M.G., 2018. Substitutability of nicotine alone and an electronic cigarette liquid using a concurrent choice assay in rats: A behavioral economic analysis. *Drug Alcohol Depend.* 185, 58–66. doi:10.1016/j.drugalcdep.2017.12.008
- Smith, J.W., Stolerman, I.P., 2009. Recognising nicotine: the neurobiological basis of nicotine discrimination. *Handb. Exp. Pharmacol.* 192, 295–333. doi:10.1007/978-3-540-69248-5_11
- Sofuoglu, M., Dudish-Poulsen, S., Nelson, D., Pentel, P.R., Hatsukami, D.K., 1999. Sex and menstrual cycle differences in the subjective effects from smoked cocaine in humans. *Exp. Clin. Psychopharmacol.* 7, 274–83. doi:10.1037/1064-1297.7.3.274
- Sofuoglu, M., & LeSage, M. G. (2012). The reinforcement threshold for nicotine as a target for tobacco control. *Drug and alcohol dependence*, 125(1-2), 1-7.
- Son, Y., Wackowski, O., Weisel, C., Schwander, S., Mainelis, G., Delnevo, C., Meng, Q., 2018. Evaluation of E-Vapor Nicotine and Nicotyrine Concentrations under Various E-Liquid Compositions, Device Settings, and Vaping Topographies. *Chem. Res. Toxicol.* 31, 861–868. doi:10.1021/acs.chemrestox.8b00063
- Spear, L. P., 2013. Adolescent neurodevelopment. *J. Adol. Health.* 52, S7-S13. PMID: 23332574.
- Spear, L.P., 2015. Adolescent alcohol exposure: Are there separable vulnerable periods within adolescence? *Physiol. Behav.* 148, 122–30. doi:10.1016/j.physbeh.2015.01.027
- Stålhandske, T., Slanina, P., 1982. Nicotyrine inhibits in vivo metabolism of nicotine without increasing its toxicity. *Toxicol. Appl. Pharmacol.* 65, 366–372. doi:10.1016/0041-008X(82)90382-9
- Swalve, N., Smethells, J.R., Carroll, M.E., 2016a. Sex differences in the acquisition and maintenance of cocaine and nicotine self-administration in rats. *Psychopharmacology (Berl)*. 233, 1005–1013. doi:10.1007/s00213-015-4183-8
- Swalve, N., Smethells, J.R., Carroll, M.E., 2016b. Sex differences in attenuation of nicotine reinstatement after individual and combined treatments of progesterone and varenicline. *Behav. Brain Res.* 308, 46–52. doi:10.1016/j.bbr.2016.04.023
- Talih, S., Salman, R., El-Hage, R., Karam, E., Karaoghlanian, N., El-Hellani, A., Saliba, N., Shihadeh, A., 2019. Characteristics and toxicant emissions of JUUL electronic cigarettes. *Tob. Control* 28, 678–680.
- Trehy, M.L., Ye, W., Hadwiger, M.E., Moore, T.W., Allgire, J.F., Woodruff, J.T., Ahadi, S.S., Black, J.C., Westenberger, B.J., 2011. Analysis of electronic cigarette cartridges, refill solutions, and smoke for nicotine and nicotine related impurities. *J. Liq. Chromatogr. Relat. Technol.* 34, 1442–1458. <https://doi.org/10.1080/10826076.2011.572213>
- Venniro, M., Caprioli, D., Shaham, Y., 2016. Animal models of drug relapse and craving: From drug priming-induced reinstatement to incubation of craving after voluntary abstinence, 1st ed, *Progress in Brain Research*. Elsevier B.V. doi:10.1016/bs.pbr.2015.08.004
- Vetter-O'Hagen, C.S., Spear, L.P., 2012. Hormonal and physical markers of puberty and their relationship to adolescent-typical novelty-directed behavior. *Dev. Psychobiol.* 54, 523–535. doi:10.1002/dev.20610
- Vieira-Brock, P. L., Andrenyak, D. M., Nielsen, S. M., Fleckenstein, A. E., & Wilkins, D. G. (2013). Age-related differences in the disposition of nicotine and metabolites in rat brain and plasma. *nicotine & tobacco research*, 15(11), 1839-1848.
- Von Weymarn, L. B., Thomson, N. M., Donny, E. C., Hatsukami, D. K., & Murphy, S. E. (2016).

Quantitation of the minor tobacco alkaloids nornicotine, anatabine, and anabasine in smokers' urine by high throughput liquid chromatography–mass spectrometry. *Chemical research in toxicology*, 29(3), 390-397.

Wada, E., Kisaki, T., Saito, K., 1959. Autoxidation of nicotine. *Arch. Biochem. Biophys.* 79, 124–130. doi:10.1016/0003-9861(59)90385-6

Wang, T., Han, W., Wang, B., Jiang, Q., Solberg-Woods, L.C., Palmer, A.A., Chen, H., 2014. Propensity for social interaction predicts nicotine-reinforced behaviors in outbred rats. *Genes, Brain Behav.* 13, 202–212. doi:10.1111/gbb.12112

Woodward, C.F., Haines, P.G., 1944. Pyrolysis of nicotine to myosmine. *J. Am. Chem. Soc.* 66, 911–914.

Zhan, Y., Liu, R., Li, Q., Leischow, S.J., Zeng, D.D., 2017. Identifying topics for e-cigarette user-generated contents: A case study from multiple social media platforms. *J. Med. Internet Res.* 19, 1–16. doi:10.2196/jmir.5780

Zhang, Y., Huo, M., Zhou, J., Xie, S., 2010. PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. *Comput. Methods Programs Biomed.* 99, 306–314. <https://doi.org/10.1016/j.cmpb.2010.01.007>