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# Sex differences in predicting nicotine-induced behavioral sensitization from habituation to novelty and initial drug response

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Sex differences in predicting nicotine-induced behavioral sensitization from habituation to novelty and initial drug response

by

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# DEDICATION

The current thesis is dedicated to the people who support my greatest aspirations and embolden me to try for more.

# ACKNOWLEDGEMENTS

Thank you to my committee members and to my other mentors who have provided me the space to learn and who have guided my growth as a scientist thus far.

# ABSTRACT

Administration of nicotine evokes an immense mesolimbic dopamine response that progressively increases, or sensitizes, with repeated drug exposure and can be monitored indirectly through rodent's motor activity. Sex differences in observed rates of behavioral sensitization in rodents appear to be consistent with epidemiological reports of smoking in humans, which indicate that females are more sensitive to the repeated effects of nicotine. Sex differences in sensitization to nicotine may explain why females progress towards addiction faster than males and so in order to effectively treat and prevent nicotine use in vulnerable populations, it is necessary to identify other factors that can be measured both before and after nicotine exposure to better predict the vulnerability of addiction. The current experiment utilized stepwise regression methods to determine if rodents' biological sex (male = 41, female = 41), rate of habituation to novelty, and initial hypoactive response to nicotine contribute to predicting the expression of behavioral sensitization after 21 days of once daily intravenous nicotine (0.05 mg/kg) injection. Reductions in the rate of habituation to a novel chamber were predictive of increased horizontal activity sensitization in female rats and may contribute to sex differences in the observed rate of horizontal activity sensitization. Female rats also displayed a reduced sensitivity to the stimulant effects of repeated nicotine on center entries. However, sex differences in center entry and center time sensitization were not related to differences in rate of habituation prior to nicotine exposure. Finally, a reduced hypoactive response to early nicotine exposure predicted greater sensitivity to the stimulant effects of nicotine on horizontal

activity in both sexes. Identifying individuals vulnerable to nicotine addiction prior to or after early drug use may be possible through combining behavioral, physiological, and neural measures and is essential to preventing adverse drug-related health outcomes.

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# LIST OF ABBREVIATIONS

CNS..... Central Nervous System

DA.....Dopamine

IP .....Intraperitoneal

IV .....Intravenous

NAc .....Nucleus Accumbens

nAchR .....Nicotinic Acetylcholine Receptor

VTA .....Ventral Tegmental Area

# CHAPTER 1

# INTRODUCTION

Cigarette smoking causes damage to nearly every organ of the body. Approximately 16 million Americans currently live with a smoking-related disease (U. S. Department of Health and Human Services (USDHHS), 2014) and approximately six million preventable deaths each year are caused by smoking (World Health Organization, 2018). In the United States, rates of cigarette smoking have declined over the past 50 years (USDHHS, 2014). However, other nicotine inhalation methods such as e- cigarettes are gaining popularity. It is intriguing that younger smokers may not use e- cigarettes to subside preexisting addictions to more harmful tobacco products, as indicated by reports from the CDC that, in 2015, 40% of e- cigarette users aged 18- 24 had never been regular users of traditional cigarettes (CDC, 2018). Comparatively, 58.8% of adults using e- cigarettes had also used traditional cigarettes in the past, with only 11.4% having never used traditional cigarettes (CDC, 2018). The prevalence of e-cigarette smoking without previous experience with traditional cigarettes indicates that youth especially may view e- cigarette smoking as a low- risk activity. However, nicotine is the primary compound of tobacco smoke believed to mediate its abuse liability and repeated exposures has profound effects on central nervous system (CNS) functioning even in the absence of some harmful substances found in cigarette products. With approximately 4.3% of middle school students and 11.3% of high school students in 2016 having used e- cigarettes in the past 30 days (CDC, 2018), the

effects of nicotine on the CNS and the neural mechanisms by which nicotine dependence develops are still highly relevant.

Robinson & Berridge (1993) describe three processes that occur with repeated drug use and drive addictive behavior. First, drug users experience craving for the drug. Then the persistence of drug craving leads to relapse. Finally, while "wanting" for the drug increases with repeated use, drug "liking" decreases with repeated use. This third point demonstrates that drugs of abuse continue to influence behavior even after the drug loses its reinforcing value. Thus, while addiction is frequently thought of as an inability to resist extraordinarily rewarding substances, nonassociative learning that occurs with repeated drug exposure renders users insensitive to the rewarding products of the drug while Pavlovian learning compels users to continue to seek drugs despite dissipating reinforcement. Alterations to circuits involved in reward, motivation, learning, and memory via these learning mechanisms influence drug saliency as well as the strength drug-related cues hold over behavior to drive the development of addiction.

Activity of the mesolimbic circuit, comprised of the ventral tegmental area (VTA) and nucleus accumbens (NAc), determines the salience of rewarding stimuli such as drugs of abuse. Alterations to the mesolimbic circuit that occur as a function of nicotine exposure are thus key to the development of nicotine dependence. Mesolimbic DA release provoked by nicotine exposure is directly mediated by nicotinic acetylcholine receptors (nAchRs). Specifically, α4β2 nAchRs are expressed by DA neurons throughout the entire VTA. However, α4α6β2 nAchRs expressed predominantly in the posterior VTA mediate the reinforcing effects of nicotine to a greater degree than other nAchRs (Pistillo et al., 2015). Consistent with reports of rats self- administering nicotine to the posterior VTA but not the

anterior VTA (Ikemoto et al., 2006), DA release in response to nicotine likely emerges primarily from cells in the posterior VTA. Generally, rewarding stimuli evoke dopamine (DA) release from the VTA to the NAc to guide learning about rewarding stimuli and contexts and to ultimately promote survival. However, nicotine and other drug- induced stimulation of DA transmission in the NAc does not undergo long- lasting habituation as seen in response to repeated exposures to non- drug rewards. Although DA transmission induced by drugs of abuse undergoes acute tolerance, normal responses return only 3 hours after exposure, compared to acute tolerance to non- drug rewards which can last anywhere from 2-24 hours (Di Chiara, 2000). This may be because CNS systems are directly stimulated by drugs but indirectly stimulated by non- drug rewards subsequent to peripheral sensory receptor stimulation (Di Chiara, 2000). A nonassociative learning process that occurs in conjunction with habituation, neural sensitization, is observed as a progressive increase in the response to nicotine as a product of changes to the mesolimbic circuit that occur with repeated exposures. For example, the expression of nAchRs in the striatum increases with repeated exposure to nicotine, rendering this region sensitive to future nicotine- induced DA release (Fung & Lau, 1988). Thus, the absence of lasting habituation and the influence that repeated nicotine has on the mesolimbic system are responsible for neural sensitization, an important process which drives drug salience.

In addition to nicotine's direct actions on DA cells; glutamate and GABA functions are targeted to manipulate mesolimbic DA transmission indirectly. Glutamate transmission to the VTA, elicited by stimulation of α7 nAchRs, provokes burst firing of VTA DA cells (Pistillo et al., 2015). GABAergic transmission, induced by stimulating α4 and β2-containing nAchRs and consequential decreases in the threshold for action potential firing

of interneurons, inhibits DA activity within the VTA (Mansvelder, Keath, & McGehee, 2002). Importantly, repeated nicotine administration provokes upregulation (Brown & Kolb, 2001) and desensitization of nAchRs on GABAergic neurons, whereas nAchRs on glutamatergic neurons do not display such responses (Mansvelder, Keath, & McGehee, 2002). As a result of nicotine- induced receptor desensitization, GABA release is diminished, disinhibiting DA activity and strengthening the ability for nicotine-induced glutamate release to stimulate further nicotine- induced DA release. Together, alterations to the mesolimbic dopamine systems allow repeatedly presented nicotine to guide extraordinarily efficient reward learning and support motivated behavior.

Lasting alterations to the input and output functions of the dorsomedial striatum (Adermark et al., 2016) as well as increased spine density in the NAc (Brown & Kolb, 2001) are some other alterations to the mesolimbic DA system that occur with repeated nicotine and have been associated with nicotine- induced motor stimulation. As such, neural sensitization of the DA response with repeated nicotine can be observed indirectly through behavioral sensitization: a consequent increase in the overt motor- stimulating effects of nicotine (Lenoir et al., 2013). Experiments administering repeated, intermediate injections of nicotine to rodents suggests that behavioral sensitization is dose- dependent (Kita, Okamoto, & Nakashima, 1991) and preserved through at least two weeks of nicotine abstinence (Clarke & Kumar, 1983; Brown & Kolb, 2001). Because behavioral sensitization occurs passively and does not require a motivated response from the animal, the dose- dependent and longstanding characteristics of nicotine- induced behavioral sensitization are likely also true of nicotine- induced DA sensitization. Behavioral sensitization does not index increased motivation but instead the neural learning processes

which occur with repeated nicotine exposures (Groves & Thompson, 1970) to increase the salience of nicotine and related stimuli (Robinson & Berridge, 1993). Increases in drug salience are associated with reports of "wanting" which may manifest as obsessive craving in experienced smokers (Robinson & Berridge, 1993). As such, behavioral sensitization may indicate greater stimulus- control over behaviors that can contribute to compulsive drug- taking that characterizes addiction (Robinson & Berridge, 1993).

Stimulation of nAchRs in the periphery (i.e. in the lungs, oral cavity, and circulatory system) potentially become associated with drug effects in the CNS (Lenoir et al., 2013) to contribute to nicotine- induced neural and behavioral sensitization. However, the behavioral and neural effects of activating nAchRs in the periphery alone are transient, habituating with repeated nicotine (Lenoir et al., 2013). As such, nicotinic actions in the periphery likely do not sensitize *per se* but may act as conditioned cues contributing to neural and behavioral sensitization. Locomotor behavioral sensitization has thus been a tool to gauge the progression of neural alterations that occur with repeated nicotine administration and to aid in uncovering the processes by which nicotine addiction develops.

Behavioral responses to nicotine vary considerably between male and female rats and suggest that the biological response to nicotine differs between the sexes. Compared to males, horizontal activity and rearing behaviors sensitize to a greater degree in female rats following 14 or 21 days of intravenous (IV) nicotine (Booze et al., 1999; Harrod et al., 2004). Sex- dependent increases in the expression of D1/ D2 receptors, which promote motor activity, or decreases in the expression of D3 receptors, which inhibit motor activity, could both contribute to greater expression of motor sensitization in females. The results of Harrod et al., (2004) support that D3 receptor expression is related to the expression of

sex differences, with females exhibiting a reduction in D3 receptor proteins and enhanced behavioral sensitization with repeated nicotine. Additionally, the distribution or metabolism of nicotine may be altered in the presence of gonadal hormones to contribute to sex differences, as suggested by results of Harrod et al., (2007) indicating concentrations of plasma nicotine in female rats, but not in ovariectomized female rats, are higher than that in males chronically exposed to nicotine.

Sex differences in nicotine- induced sensitization have also been identified using dependent measures of centrally- directed activity (Harrod et al., 2004). Importantly, sex differences in sensitization of centrally- directed activities exhibit different patterns than sensitization of general activity measures. Fear and emotionality prior to and in the presence of repeated nicotine are also sex- dependent and likely contribute to drug sensitivity via connections between the central amygdala and the NAc shell (Zarrindast et al., 2012). Accordingly, observed centrally- directed activity in rats with drug exposure have been used to index sex- dependent alterations to the anxiolytic/anxiogenic effects of nicotine. In an elevated plus maze paradigm, female rats are more sensitive to the anxiogenic effects of orally- administered nicotine than male rats (Caldarone, King, and Picciotto, 2008). Cao et al., (2010) similarly reported an anxiogenic effect of nicotine only in adult female rats, with nicotine significantly reducing the time in the center of the chamber and also provoking enhanced corticosterone release. Therefore, centrally-directed measures of locomotor activity may be of particular interest to scientists exploring the influence of repeated nicotine on behaviors which index stress and are critical to understanding if stress- related processes indeed promote higher rates of nicotine dependence in females compared to males (Torres and O'Dell, 2016).

The results of experiments studying behavioral sensitization in male and female rats are consistent with what is reported in humans. Women progress towards addiction at a faster rate than men (Westermeyer & Boedicker, 2000) and report higher anxiety than men during nicotine abstinence (Torres & O'Dell, 2016). These reports indicate that women may be more vulnerable to the actions of nicotine which provoke drug- context learning and stressful withdrawal. More so, women administered various doses of intranasal nicotine (0, 5, 10, 20 μg/kg) were less responsive to the discriminant stimulus effects of nicotine (Perkins, 1999) supporting that sex differences are a product of CNS processes, rather than a product of differences in the peripheral response to nicotine. Despite these advances in our understanding of how nicotine addiction develops, it is still largely unexplored how behaviors prior to nicotine exposure or soon after initial nicotine exposure may predict sensitivity to help identify populations vulnerable to repeated nicotine.

The behavioral consequences of neural learning processes other than sensitization, and that are influenced by repeated drug exposure, may provide further clarification on the processes that contribute to addiction in vulnerable individuals. Habituation of neural responses can be indirectly observed through progressive decreases in the behavioral response to a stimulus. It is feasible that animals less capable of habituating to a non- drug stimulus may also be less capable of habituating to a drug such as nicotine. Although previous experiments have considered whether an animal's *initial locomotor response* to novelty is related to addiction- like behaviors later on (see discussion: Deroche et al., 1993; Bevins & Besher, 2001; Coolon & Cain, 2009; Nishida et al., 2016), the *rate of habituation* to novelty over two sessions has not yet been examined as a potential predictor of drug

sensitivity. Because habituation to the depressive effects of nicotine occurs simultaneously with sensitization of the stimulant effects of nicotine, the rate at which an animal habituates to a novel stimulus likely impacts the observed expression of behavioral sensitization. For example, if the initial depressive effects of nicotine on behavior are driven by an initial increases in GABA release, desensitization of nAchRs may be the mechanism behind rapid behavioral habituation to these effects. This hypothetical slowing of nAchR desensitization would prevent habituation of nicotine- induced GABA release and inhibit the release or influence nicotine- induced DA transmission. Interestingly, estrogen may act on striatal GABA to influence females' response to nicotine (Becker, 1999) and possibly the rate at which they habituate to an environment. More so, it has been suggested that external drug cues modulate drug maintained relapse in women to a greater degree than in men (Perkins et al., 2001), indicating the rate at which rats habituate to an external stimulus prior to nicotine may serve to predict sex differences in the rate of behavioral sensitization with repeated nicotine exposures.

Examining behavioral responses to the initial depressive effects of nicotine may also contribute to understanding the variability in an individual's sensitivity to develop addiction with repeated exposures. An experiment by Davidson, Finch, and Schenk, (1993) suggested that the initial positive effects of cocaine use were negatively correlated with latency to next use and positively correlated with lifetime use in 80 undergraduate students. While this experiment also reports that the negative effects of initial cocaine use, analogous to the depressive effects of initial nicotine, did not significantly correlate with latency or lifetime use, the stimulant effects of nicotine following initial use are difficult to observe in animals due to acute nicotine's hypoactive effects. Nicotine- induced depression of

behavior, however, is most apparent in animals approximately 15- 20 minutes following acute nicotine (Morrison & Stephenson, 1972) and, like nicotine- induced stimulation of behavior, is related to nucleus accumbens function (Kita, Okamoto, & Nakashima, 1991). Habituation to nicotine- induced hypoactivity (Clarke & Kumar, 1983) develops in rodents within two to five 0.4 mg/kg subcutaneous injections (Morrison & Stephenson, 1972) or one 1.0 mg/kg intraperitoneal (IP) injection of nicotine and persists through at least 90 days of abstinence from IP- administered nicotine (Stolerman, Fink, & Jarvik, 1973). According to Morrison & Stephenson (1972), habituation to the motor- depressing effects of nicotine in rats may parallel the development of tolerance to the negative effects of smoking (i.e. nausea or sweating) reported by novice smokers. Thus, describing both the stimulant and depressive effects of nicotine on motor behavior may inform researchers about DA activity in the NAc.

Understanding nicotine- induced sensitization of the mesolimbic DA system and locomotor behaviors are necessary to develop effective treatments for those dependent on nicotine. However, to *prevent* vulnerable individuals from becoming dependent on nicotine, identifying characteristics that can be observed prior to or at early drug exposures and contribute to nicotine- induced sensitization are necessary. The primary aim of the current experiment was to determine if locomotor behavior prior to or following acute nicotine exposure could contribute to predicting an animal's expression of behavioral sensitization following repeated nicotine. Stepwise regression methods were used to determine if biological sex, rates of habituation prior to nicotine exposure, or hypoactivity following acute nicotine were predictive of the rate of behavioral sensitization. A main effect of biological sex was expected to be included in each final model, as females are

expected to show greater rates of behavioral sensitization. It was hypothesized that lower (less negative/more positive) rates of habituation would predict high rates of behavioral sensitization following repeated nicotine. However, the potential to predict horizontal activity sensitization from habituation rates may differ from that of centrally- directed behavior sensitization. If the rate of habituation to a novel chamber indexes differences in stress- related processes, sensitization of centrally- directed behaviors would be expected to be more sensitive to the influence of habituation than sensitization of general activity measures. Further, the interaction term was expected to take on a greater predictive value in models considering centrally- directed behaviors due to previous results indicating that centrally- directed behaviors are used in other paradigms (i.e. elevated plus maze and open field) to index anxiolytic drug effects and biological sex differences in the anxiolytic/anxiogenic effects of nicotine (Caldarone, King, & Picciotto, 2008). Successful prediction of sensitization of either horizontal or centrally- directed activities has substantial implications for the future of identifying and treating individuals vulnerable to nicotine addiction.

# CHAPTER 2

# METHODS

The data described in the current experiment has been analyzed previously as described by Illenberger et al., (2018) to test hypotheses regarding the testing chamber's influence on biological sex differences, hypoactivity, and behavioral sensitization. In the current experiment, chamber shape will be the first independent variable entered into each model so that variability attributable to chamber shape will not be inappropriately attributed to one of the variables of interest. However, results specific to the factor of chamber shape will not be discussed in great detail as the significance of testing chamber shape on activity and sex differences following nicotine has been established and discussed (Illenberger et al., 2018). The current analyses will instead test and discuss the novel hypotheses stated above. Protocols for this research were approved by the Institutional Animal Care and Use Committee (IACUC; Assurance number D16-00028).

## 2.1 Animals/ Surgeries

41 male and 41 female Sprague- Dawley rats received surgical implants of intracath IV catheters at Harlan Laboratories, Inc. according to the procedures of Mactutus et al., (1994). Catheters were secured into each animal's jugular vein and the subcutaneous port was implanted on the dorsal surface to ease daily IV administration.

## 2.2 Drug Administration

Daily nicotine was administered via the IV route because it produces a rapid influx of arterial nicotine that mimics the pharmacokinetic profile of nicotine absorption from cigarette smoking (Benowitz, 1988). For each injection, 0.05 mg/kg of saline or nicotine were administered over a 15- sec duration. To maintain catheter patency, all IV injections were followed by an injection of 0.2 ml heparinized (2.5%) saline, also delivered over a 15- sec duration. Due to a loss of catheter patency across the experiment, four male rats and three female rats were excluded from the final analysis.

## 2.3 Behavioral Testing

Upon recovery from catheter surgery, animals were shipped to the animal care facilities at the University of South Carolina and arrived at approximately 90 days of age. After seven days of quarantine, animals were transferred to the colony room maintained at 21 ± 2˚C, 50 ± 10% relative humidity. Lights were turned on at 07:00 AM daily for a 12 hour period and rodent food (Pro- Lab Rat, Mouse Hamster Chow #3000) and water were available ad libitum.

Locomotor activity testing occurred in Hamilton- Kinder activity chambers under dim lighting conditions. Testing chambers were constructed with clear Plexiglas to create a square or round field design. The chamber manufacturer tuned the 32 photocells approximately 4 cm above the chamber floor to account for round chamber shapes. Photobeam breaks and restorations as a result of animal movement were recorded automatically by computer and translated into counts per each five minute interval of one

hour testing sessions. The dependent measure horizontal activity refers to the sum of beam breaks recorded in X- and Y- planes parallel to the chamber floor. Center entries refer to the number of times an animal crossed into the center 25% of the chamber whereas center time refers to the total time (sec) spent within the center 25% of the chamber.

Animals were placed in locomotor activity chambers for testing on five occasions. The first two sessions allowed animals to habituate to the testing chamber without prior administration of saline or nicotine. Prior to the third testing session, however, 0.05 mg/kg IV saline was administered to each animal. Locomotor activity observed following saline injection served as a baseline measure by which to compare activity following later nicotine administration. The first of 21 total 0.05 mg/kg IV nicotine injections was administered prior the fourth testing session to determine the rate of horizontal activity depression that occurred with acute nicotine. To ensure that behaviors observed following the final ($21^{st}$) injection are not attributable to responses to a conditioned stimulus (i.e. the testing chamber) animals were returned to their home cages rather than placed in chambers for testing following the $2^{nd}$- $20^{th}$ nicotine injection. As such, the fifth testing session, to determine the influence of repeated nicotine on horizontal activity and centrally- directed behaviors, was not conducted until after the $21^{st}$ IV nicotine injection.

## 2.4 Statistical Analysis

All analyses were conducted in SPSS 24 (IBM Software). Behavioral sensitization, rate of habituation, and hypoactivity were calculated as shown by the formulas displayed in **Appendix A**. Behavioral sensitization, the dependent variable in each model, was calculated by subtracting the amount of activity following saline injection from the amount

of activity following the 21st nicotine injection (Nic21 - Sal). The behavioral rate of changes that served as independent variables were calculated in a similar manner. The rate of habituation was calculated by subtracting the amount of activity on the first habituation day from the amount of activity on the second habituation day (Hab2 - Hab1) and the rate of hypoactivity was calculated by subtracting the amount of activity following saline injection from the amount of activity following the first nicotine injection (Nic1 - Sal). Using difference scores rather than raw scores of observed behavior ensures that observed differences in activity on a given day do not affect the estimates used as predictors of behavioral sensitization. Continuous variables were mean centered prior to analysis and the means and the standard error of the means are reported as $\bar{X} \pm SEM$.

Stepwise regressions were used to test if the expression of behavioral sensitization can be predicted from biological sex and habituation rates prior to nicotine exposure, or from biological sex and hypoactivity at the first exposure to nicotine. Criteria was set to an F value of at least 1.25 to enter the regression model, and an F value of at least 1.0 to stay in the model for subsequent steps. The intercept was not included in any of the models. Adjusted R square or change in R square values are presented for each step of the four stepwise regressions. Main effect terms for chamber shape, biological sex, and rate of habituation or rate of hypoactivity, as well as the interaction term between biological sex and habituation or hypoactivity were considered as potential predictors.

The rate of behavioral sensitization, habituation, or hypoactivity can theoretically be derived from any of the behaviors observed during testing (including basic and fine movements, distance travelled, and rearing). However, experiments from both ours and other laboratories have revealed that the expression of biological sex differences and

differences in rates of sensitization depend on the behavioral measure under consideration (Booze et al., 1999; Cao et al., 2010; Illenberger et al., 2018). Consequently, the expression of habituation, hypoactivity, and sensitization was expected to vary considerably across measures of general activity and centrally - directed activities despite being recorded from a single animal within the same testing session. As such, the current analysis derived change in activity scores from the total observed horizontal activity, entries into the center (center entries), and time spent in the center (center time) throughout one hour testing sessions and each of the three observed behaviors were considered in separate models. Correlations between the three dependent variables (i.e. behavioral sensitization of horizontal activity, center entries, and center time) are displayed in **Table 2.1**.

The first stepwise regression determined if biological sex, the rate of horizontal activity habituation prior to nicotine exposure, and/or the interaction between these variables contribute to predicting sensitization of horizontal activity. It was hypothesized females would display lower (less negative/more positive) rates of habituation than males and that low rates of habituation would predict high rates of behavioral sensitization following repeated nicotine. The second and third stepwise regression models considered behaviors measured by centrally - directed behaviors rather than horizontal activity. Biological sex, the rate of habituation as measured by center entries or center time, and the interaction term were included in the models predicting expression of center entry or center time sensitization, respectively. The final regression modeled the expression of horizontal activity sensitization once again. However, the rate of hypoactivity following initial exposure to nicotine, rather than the rate of habituation prior to nicotine, will be considered as a predictor in addition to biological sex and the interaction term.

Table 2.1 Pearson correlations between vectors of values of sensitization as measured by horizontal activity, center entries, and center time. Asterisks indicate that the correlation is significant at the 0.01 level (2-tailed).

| **Pearson correlations between vectors of values** | | | |
|---|---|---|---|
| | **Horizontal Activity Sensitization** | **Center Entry Sensitization** | **Center Time Sensitization** |
| **Horizontal Activity Sensitization** | 1.000 | 0.520* | 0.313* |
| **Center Entry Sensitization** | --- | 1.000 | 0.656* |
| **Center Time Sensitization** | --- | --- | 1.000 |

# CHAPTER 3

## RESULTS

The first stepwise regression procedure tested whether significant quantities of variability in nicotine- induced horizontal activity sensitization can be attributed to biological sex and/or the rate of habituation to a novel testing chamber. The scatterplot conveying how horizontal activity sensitization relates to horizontal activity habituation in male and female rats is displayed in **Figure 3.1**. The average rates of horizontal activity habituation and sensitization prior to mean centering were -473.09 ± 37.63 ambulations and 168.00 ± 31.70 ambulations, respectively. As planned, the first step tested whether the shape of the chamber significantly contributes to the prediction of horizontal activity sensitization in male rats exposed to repeated IV nicotine injections. Testing chamber shape did not provide significantly greater prediction of sensitization in males compared to predicting sensitization from the mean sensitization rate alone (R square change = 0.025; $F(1,74) = 1.928$, $p \leq 0.169$). Biological sex explained an additional 4% of the variance in horizontal activity sensitization and significantly improved the model, increasing the adjusted R square to 0.065 (R square change = 0.065; $F(1,73) = 5.182$, $p \leq 0.026$). The third step entered the interaction term between biological sex and the rate of habituation to novelty into the model, explaining an additional 4% of the variation in behavioral sensitization. The ability to predict behavioral sensitization was significantly improved and the adjusted R square increased to 0.1 (R square change = 0.049; $F(1,72) = 4.113$, $p \leq 0.046$). The main effect for rate of horizontal activity habituation to novelty was the only

## Scatterplot of horizontal activity habituation and sensitization

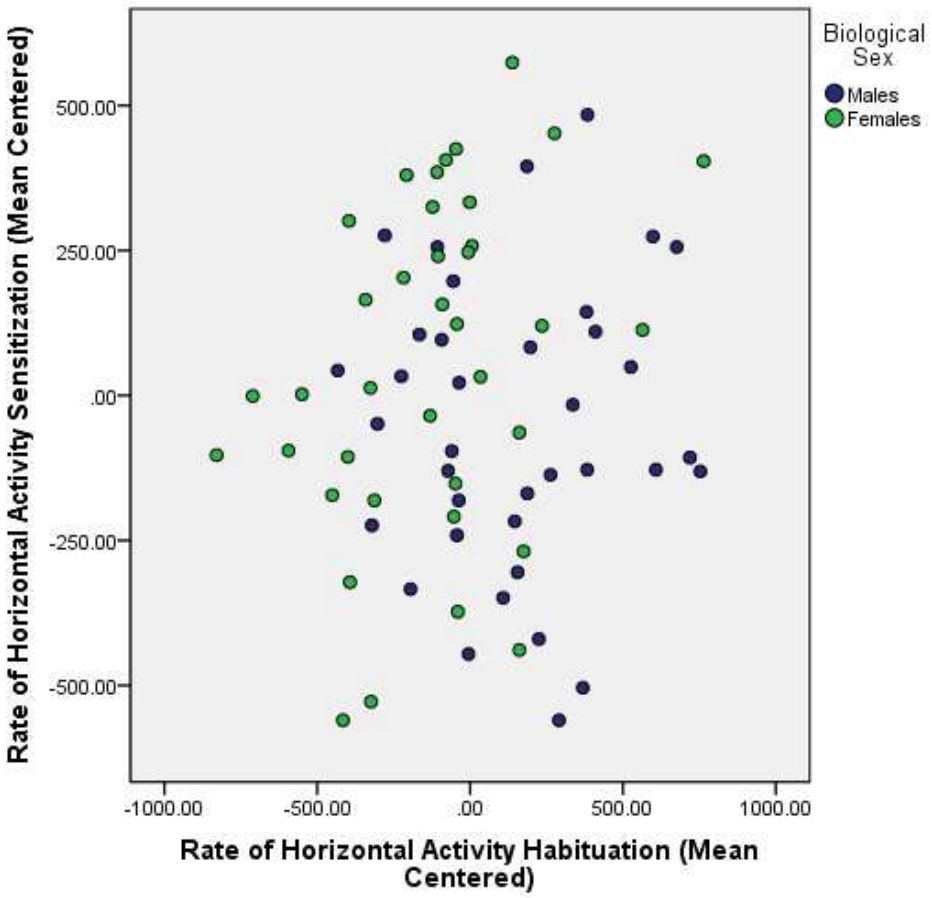


Figure 3.1 Scatterplot of horizontal activity habituation and sensitization in male and female rats. Rates of habituation and sensitization were mean centered so that values of zero on the axes correspond to average rates of habituation or sensitization, respectively.

variable excluded from the final regression model of the current procedure. Chamber shape, biological sex, and the interaction between biological sex and the rate of habituation all significantly contributed to predicting horizontal activity sensitization. The B coefficient, standard error of the B coefficient, β coefficient, t- value, significance values, and partial correlation coefficients are presented in greater detail in **Table 3.1** for each variable entered into the three models of horizontal activity sensitization considered in the stepwise regression. The β coefficient for biological sex indicates that females displayed higher rates of horizontal activity sensitization, consistent with the implications of Illenberger et al., (2018). The β coefficient for the interaction term indicates that the slope of the regression line is significantly higher for female rats compared to male rats. Further, exclusion of the main effect for rate of horizontal activity habituation indicates that the slope of the regression line for males is not significantly different from zero. Consistent with our hypotheses, these results suggest that, in female rats, reduced habituation to novelty prior to nicotine exposure may be predictive of high rates of horizontal activity sensitization with repeated nicotine.

The second stepwise regression procedure tested whether biological sex and/or the rate of center entry habituation to novelty significantly contributes to predicting nicotine-induced sensitization of center entries. **Figure 3.2** displayed the relationship between center entry habituation and sensitization in male and female rats. The average rate of center entry habituation and sensitization prior to mean centering were -46.98 ± 6.74 center entries and 79.09 ± 7.58 center entries, respectively. Consistent with the results of Illenberger et al., (2018), testing chamber shape significantly contributed to the prediction of center entry sensitization beyond prediction from the mean alone. Approximately 6%

Table 3.1 The first stepwise regression completed in three steps. Only the term for the main effect of habituation rate was not entered into the model. The final model had an adjusted R square of 0.103 and included chamber shape, biological sex, and the interaction between biological sex and habituation rate.

| **Models predicting horizontal activity sensitization from habituation** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Model** | **Included Variables** | **Unstandardized B Coefficient** | **Standard Error** | **Standardized (Beta) Coefficients** | **t** | **Sig.** | **Partial Correlation Coefficient** |
| **1** | **Chamber Shape** | -61.184 | 44.062 | -0.159 | -1.389 | 0.169 | -0.159 |
| **2** | **Chamber Shape** | -117.526 | 49.499 | -0.306 | -2.374 | 0.02 | -0.268 |
| | **Biological Sex** | 112.684 | 49.499 | 0.293 | 2.276 | 0.026 | 0.257 |
| **3** | **Chamber Shape** | -107.581 | 48.724 | -0.28 | -2.208 | 0.03 | -0.252 |
| | **Biological Sex** | 142.024 | 50.589 | 0.37 | 2.807 | 0.006 | 0.314 |
| | **Sex X Habituation Rate** | 0.27 | 0.133 | 0.24 | 2.028 | 0.046 | 0.232 |

## Scatterplot of center entry habituation and sensitization

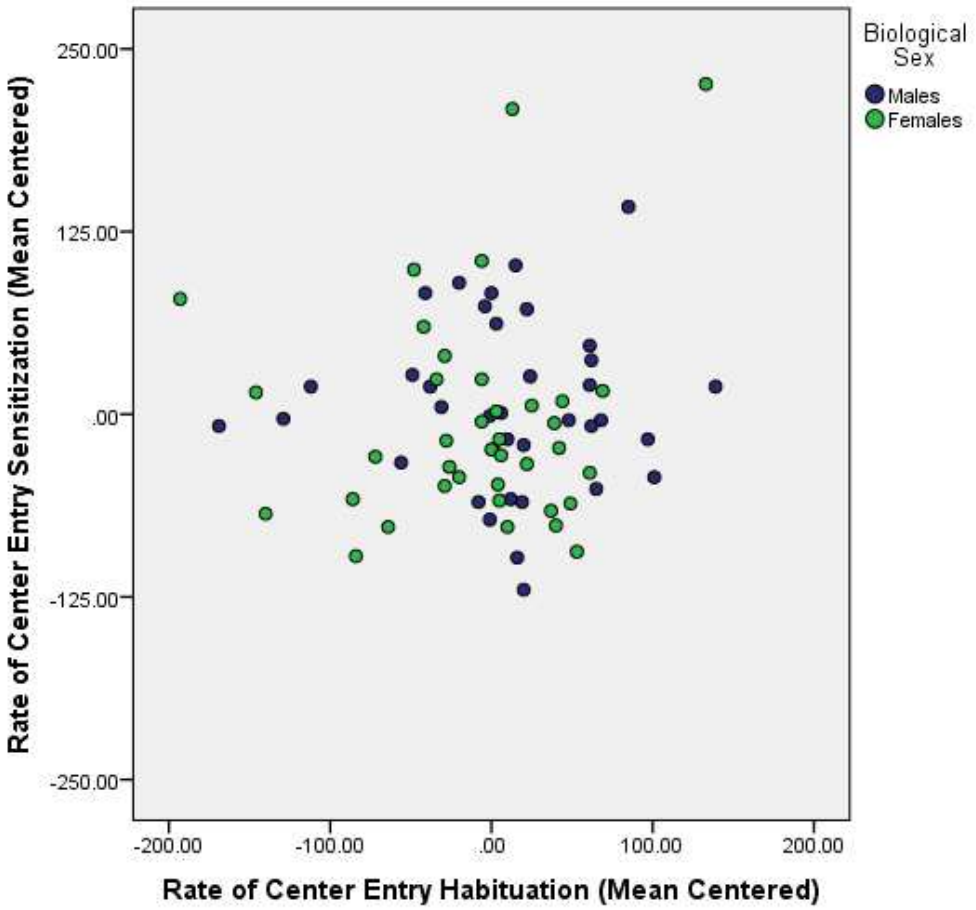


Figure 3.2 Scatterplot of center entry habituation and sensitization in male and female rats. Rates of habituation and sensitization were mean centered so that values of zero on the axes correspond to average rates of habituation or sensitization, respectively.

of the variance in center entry sensitization in males was attributable to chamber shape (R square change = 0.07; $F(1,74) = 5.579$, $p \leq 0.021$). An additional 5% of the variance in center entry sensitization was attributable to considering biological sex in the second step of the procedure. Including biological sex significantly increased the adjusted R square from 0.058 to 0.097 (R square change = 0.051; $F(1,73) = 4.271$, $p \leq 0.042$). The interaction term between biological sex and the rate of center entry habituation was added to the model in the third and final step of the current procedure. Approximately 2% of the variance in center entry sensitization was explained by including the interaction term, however, this did not significantly improve the models prediction of center entry sensitization compared to the previous step (R square change = 0.024; $F(1,72) = 2.015$, $p \leq 0.16$). The final model, including testing chamber shape, biological sex, and the interaction term, excluded only the main effect for the rate of center entry habituation. Testing chamber shape and biological sex significantly contributed to the model's prediction of behavioral sensitization and produced a final adjusted R square value of 0.11. The B coefficient, standard error of the B coefficient, β coefficient, t- value, significance values, and partial correlation coefficients are presented in greater detail in **Table 3.2**. The β coefficient for biological sex indicates that female rats displayed lower rates of center entry sensitization than male rats, also consistent with the implications of Illenberger et al (2018). Exclusion of the main effect of habituation indicates that the slope of the regression line for male rats was not significantly different from zero. An insignificant coefficient for the interaction term indicates that the slope of the regression line for female rats was not significantly different from that of males. The results of this second procedure support that the relationship between habituation to novelty and nicotine- induced sensitization are

Table 3.2 The second stepwise regression completed in three steps. Only the term for the main effect of habituation rate was not entered into the model. The final model had an adjusted R square of 0.11 and included chamber shape, biological sex, and the interaction between biological sex and habituation rate, however the interaction term did not significantly contribute to the model.

| **Models predicting center entry sensitization from habituation** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Model** | **Included Variables** | **Unstandardized B Coefficient** | **Standard Error** | **Standardized (Beta) Coefficients** | **t** | **Sig.** | **Partial Correlation Coefficient** |
| **1** | **Chamber Shape** | 24.249 | 10.266 | 0.265 | 2.362 | 0.021 | 0.265 |
| **2** | **Chamber Shape** | 36.236 | 11.601 | 0.396 | 3.124 | 0.003 | 0.343 |
| | **Biological Sex** | -23.974 | 11.601 | -0.262 | -2.067 | 0.042 | -0.235 |
| **3** | **Chamber Shape** | 39.416 | 11.737 | 0.43 | 3.358 | 0.001 | 0.368 |
| | **Biological Sex** | -23.128 | 11.536 | -0.253 | -2.005 | 0.049 | -0.23 |
| | **Sex X Habituation Rate** | 0.231 | 0.163 | 0.16 | 1.42 | 0.16 | 0.165 |

dependent on the measure of activity being considered. Habituation to novelty significantly contributed to the prediction of nicotine- induced behavioral sensitization when these responses were measured by horizontal activity but not center entries. Although not anticipated, these results are compatible with the notion that changes to centrally- directed behaviors convey different alterations to underlying neural processes than changes to general measures of activity.

The third stepwise regression tested whether sensitization of time spent in the center of the chamber with repeated nicotine could be predicted from biological sex and/or the rate of habituation prior to nicotine exposure. Additionally, it was of interest to determine if the terms included in the model predicting center time sensitization are similar to those in the model predicting center entry sensitization. The relationship between habituation to novelty and nicotine- induced sensitization as measured by male and female rats' center time is displayed in **Figure 3.3**. The average rate of center time habituation and sensitization prior to mean centering were -20.01 ± 24.98 sec and 326.01 ± 45.21 sec, respectively. Stepwise regression methods failed to produce a significant model of center time sensitization from the variables: testing chamber shape, biological sex, rate of center time habituation, and the interaction between biological sex and rate of habituation. The first step, including only testing chamber shape, did not significantly improve prediction of center time sensitization in male rats beyond prediction from the mean alone (R square change = 0.04; $F(1,74) = 3.076$, $p \leq 0.084$). None of the variables of interest provided enough predictive value to be entered into the model at the second step of the procedure. Similar to what was observed in the analysis of center entries, novelty- induced changes to the time spent in the center of the chamber were not related to nicotine- induced changes.

## Scatterplot of center time habituation and sensitization

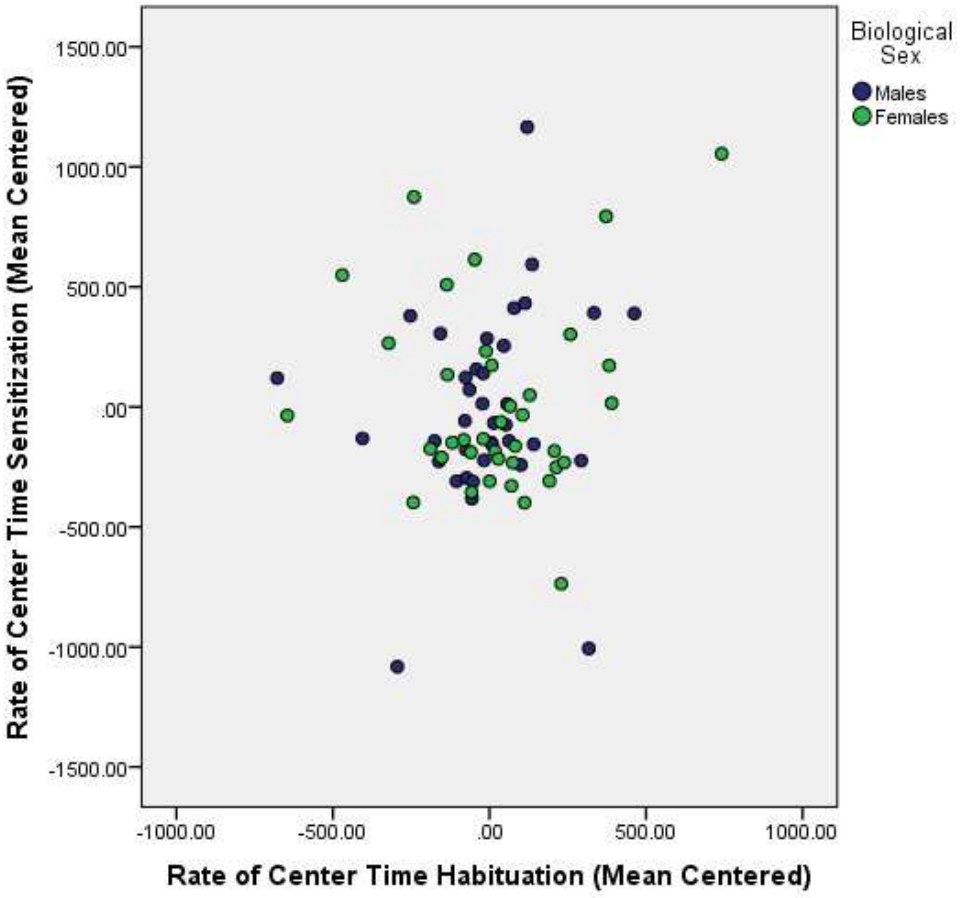


Figure 3.3 Scatterplot of center time habituation and sensitization in male and female rats. Rates of habituation and sensitization were mean centered so that values of zero on the axes correspond to average rates of habituation or sensitization, respectively.

However, there was no evidence of sex difference in the rate of center time sensitization, as was observed in horizontal activity and center entry sensitization. The final stepwise regression tested whether biological sex and/or the rate of hypoactivity elicited by initial nicotine exposure can significantly contribute to predicting nicotine- induced sensitization of horizontal activity with repeated exposures.

The final stepwise regression tested whether biological sex and/or the rate of hypoactivity elicited by initial nicotine exposure can significantly contribute to predicting nicotine- induced sensitization of horizontal activity with repeated exposures. **Figure 3.4** displays the relationship between the responses to acute and repeated nicotine, as measured by horizontal activity. The average rate of horizontal activity hypoactivity prior to mean centering was -227.10 ± 28.93 ambulations. The first step of the current stepwise regression procedure was the same as that of the first stepwise procedure in that both test whether chamber shape contributes to predicting horizontal activity sensitization. The main effect for hypoactivity of horizontal activity was entered into the model in the second step, explaining approximately 16% of the variance in horizontal activity sensitization and significantly improving the model (R square change = 0.179; $F(1,73) = 16.442$, $p \leq 0.001$). The third and final step of the current procedure included biological sex as a predictor of horizontal activity sensitization. Biological sex significantly improved the model, explaining approximately 4% of the variance in horizontal activity sensitization (R square change = 0.046; $F(1,72) = 4.451$, $p \leq 0.038$). The final model had an adjusted R square value of 0.22 and included significant contributions from testing chamber shape, hypoactivity, and biological sex. The B coefficient, standard error of the B coefficient, β

## Scatterplot of horizontal activity hypoactivity and sensitization

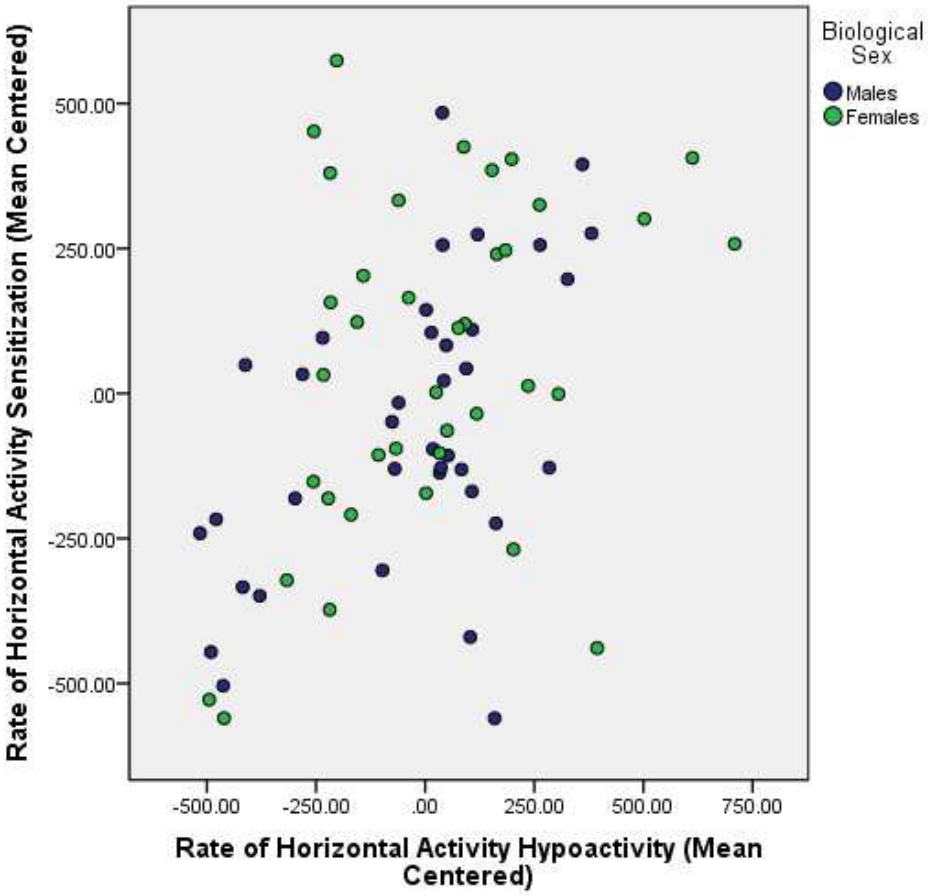


Figure 3.4 Scatterplot of horizontal activity hypoactivity and sensitization in male and female rats. Rates of hypoactivity and sensitization were mean centered so that values of zero on the axes correspond to average rates of hypoactivity or sensitization, respectively.

Table 3.3 The fourth stepwise regression completed in three steps. Only the interaction term between biological sex and hypoactivity was not entered into the model. The final model had an adjusted R square of 0.22 and included chamber shape, hypoactivity, and biological sex.

| **Models predicting horizontal sensitization from hypoactivity** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Model** | **Included Variables** | **Unstandardized B Coefficient** | **Standard Error** | **Standardized (Beta) Coefficients** | **t** | **Sig.** | **Partial Correlation Coefficient** |
| **1** | **Chamber Shape** | -61.184 | 44.062 | -0.159 | -1.389 | 0.169 | -0.159 |
| **2** | **Chamber Shape** | -47.962 | 40.211 | -0.125 | -1.193 | 0.237 | -0.138 |
| | **Hypoactivity** | 0.444 | 0.11 | 0.425 | 4.055 | < 0.001 | 0.429 |
| **3** | **Chamber Shape** | -96.514 | 45.537 | -0.251 | -2.119 | 0.037 | -0.242 |
| | **Hypoactivity** | 0.423 | 0.108 | 0.404 | 3.932 | < 0.001 | 0.42 |
| | **Biological Sex** | 95.829 | 45.425 | 0.25 | 2.11 | 0.038 | 0.241 |

coefficient, t- value, significance values, and partial correlation coefficients are presented in greater detail in **Table 3.3**. The β coefficient for hypoactivity of horizontal activity suggests that low rates of hypoactivity (more positive values) following initial nicotine predict high rates of sensitization with repeated nicotine. Also important to consider, unlike the model in which habituation to novelty was considered, the main effect term for hypoactivity was entered into the model earlier than the main effect for biological sex, indicating that the initial nicotine response may be more predictive of sensitivity to repeated nicotine than biological sex. Our hypothesis was supported in that hypoactivity following acute nicotine did significantly contribute to predicting sensitization in male and female rats. However, with low rates of hypoactivity predicting high rates of sensitization, the relationship was in the opposite direction of what was originally predicted. The β coefficient for biological sex indicates that female rats exhibited significantly greater horizontal activity sensitization than male rats. However, the relationship between horizontal activity hypoactivity and sensitization is not significantly different in male and female rats, as indicated by exclusion of the interaction term.

# CHAPTER 4

# DISCUSSION

The current experiment successfully identified behaviors which could be observed prior to and following the first nicotine exposure that significantly improved prediction of horizontal activity sensitization following repeated nicotine. Significantly, habituation to novelty contributed to the prediction of nicotine- induced horizontal activity sensitization in female, but not male rats. In contrast, the hypoactive response to nicotine contributed to the prediction of nicotine- induced horizontal activity sensitization in both males and females. The results support previous work from our lab indicating that nicotine elicits increased horizontal activity sensitization (Booze et al., 1999; Harrod et al., 2004) but decreased center entry sensitization in female rats compared to male (Illenberger et al., 2018). Additionally, the results endorse the conclusions of Illenberger et al., (2018), indicating that the underlying neural processes that contribute changes to horizontal activity, center entries, and center time are different. Collectively, the results of the current experiment demonstrate that certain behaviors related to the development of addiction can be used as early signs of vulnerability to the repeated effects of drugs and that the processes contributing to nicotine- induced sensitization of behaviors significantly depend on the measure of activity and the population under examination (i.e. males vs females).

Previous experiments examining the relationship between animals' response to novelty and vulnerability to addiction have produced mixed results when considering

different classes of drugs. Studies with morphine have demonstrated that animals with high responses to novelty exhibit increased drug- induced motor sensitization and reduced morphine- induced analgesia (Deroche et al., 1993). Interestingly, while the results of Nishida et al., (2016) contradict these findings, indicating the novelty response is not related to the effects of repeated morphine, animals in this experiment that displayed a high response to novelty also displayed higher sensitivity to the stimulant effects of acute morphine. Further, nicotine- induced hyperactivity was not related to an animal's response to a novel chamber in an experiment by Coolon and Cain, (2009) and was negatively related to novelty in an experiment by Bevins and Besheer, (2001). It is of note, however, that the experiments by Coolon and Cain, (2009) and Bevins and Besheer, (2001) only analyzed the initial response to novelty, observed during one session, and only male rats were tested. The current experiment is thus the only study to our knowledge to ask whether motor *habituation* to a novel stimulus over two sessions can predict nicotine- induced hyperactivity in male and female rats. It is also of interest for future experiments to determine if within- session rate of habituation can also contribute to predicting behavioral sensitization in rats, either alone or in addition to the information provided by across-session habituation.

The current results suggest that female rodents' rate of habituation to novelty recorded prior to nicotine exposure is related to the rate of horizontal activity sensitization displayed following repeated nicotine exposures. Female rats that showed greater habituation to the novel chamber over two sessions appear less sensitive to the stimulating effects of repeated nicotine on horizontal activity behavior. In contrast, shifts in male rodents' rate of habituation to novel chambers did not significantly influence the expression

of horizontal activity sensitization with repeated nicotine. These results indicate that different neural processes or different recruitment of the same/similar neural processes may contribute, in part, to sex differences in the expression of behavioral sensitization. Further, the proposed sex differences in neural recruitment may be the mechanism rendering female rodents more sensitive to the stimulant effects of repeated nicotine on horizontal activity.

Habituation to novelty likely contributes less to the overall potential to predict sensitization of centrally- directed behaviors because stress- related and other processes are likely recruited to a greater degree. However, a significant main effect of biological sex suggests that sex differences in the recruitment of processes outside of habituation contribute to increased center entry sensitization in male rats exposed to repeated nicotine. Consistent with previous research of behavior displayed by male and female rats in elevated plus maze (Caldarone, King, & Picciotto, 2008) and open field (Cao et al., 2010), increased centrally- directed activity with repeated nicotine exposure indicates that male rats may experience anxiolytic effects of nicotine to a greater degree than female rats. It is interesting, however, that females displayed reduced sensitization of center entry but not center time, suggesting that females may not be avoiding the center of the chamber, but instead making fewer alterations between the center and periphery. More so, the results of Harrod et al., (2004) indicate that, compared to male rats, center distance sensitization is actually increased in female rats under the same administration regimen used in the current experiment. Different results for the models considering center entry and center time support the conclusions of Illenberger et al., (2018), that measures of behavior such as horizontal activity and center entries (or in this case center entries and center time) are unique in how environmental factors, biological sex, and learning processes contribute to

their expression. However, further research will be needed to resolve the etiology of opposing sex differences across measures of centrally- directed activity. As such, multiple dependent measures should be used to explore questions regarding locomotor activity in rodents to provide the most complete interpretation of experimental effects. The results of the sensitization models for both horizontal and centrally- directed activity indicate that sex differences in habituation- related processes may contribute to greater sensitivity to the stimulant effects of repeated nicotine in females but sex differences in processes not related to habituation drive greater sensitivity to the anxiolytic effects of repeated nicotine in males.

Because initial exposures to nicotine depresses locomotor activity in drug- naïve rats, it is difficult to monitor the progression of sensitization to the stimulant effects of nicotine before tolerance to the hypoactive response develops with repeated drug exposures. However, previous reports have demonstrated that the initial effects of morphine in animals and cocaine in humans is predictive of future drug use (Nishida et al., 2016; Davidson, Finch, & Schenk, 1993). Animals that were less sensitive to the analgesic effects of initial IV morphine (2.5 mg/kg) appeared more vulnerable to addiction given continued drug access (Nishida et al., 2016). Comparatively, the positive, but not negative, subjective effects of initial cocaine use were related to shorter latencies to next use and lifetime cocaine use in undergraduate students (Davidson, Finch, & Schenk, 1993). Further, experiments in humans and animals have supported that early alcohol experiences can also be predictive of whether alcohol addiction develops with continued use (Schuckit, 1994; Schramm- Sapyta et al., 2008). The current experiment expands on this literature by demonstrating that passive sensitivity to the repeated effects of nicotine on horizontal

activity behavior is also related to the effects experienced at initial drug use in male and female rats.

Rather than passive administration methods as used in the current experiment, experiments previously examining predictors of drug sensitivity have also examined addictive- like behaviors following drug self- administration (Coolon & Cain, 2009; Nishida et al., 2016). Total drug intake and rate of intake are of interest to those exploring individual differences in how addiction develops. However, passive administration methods ensure that differences in the expression of behavioral sensitization cannot be attributed to differences in the dose of nicotine or the interval at which nicotine was self-administered. One advantage of the experimental design used by Coolon and Cain, (2009) is the ability to test whether the initial response to a novel chamber influences the conditioned locomotor effects of nicotine. Instead, the current design prevented repeated pairing of the drug effects and the context to ensure that nicotine- induced hyperactivity was a product of sensitization and not conditioned hyperactivity. The current design thus explores behavioral predictors of drug sensitivity through methods that are not well-represented in the current literature.

One limitation of the current experiment is that only one dose (0.05 mg/kg/injection) of nicotine was used. As the current literature suggests that sex differences related to nicotine sensitivity are most apparent at low doses, it is of interest to determine if the sex differences reported in the current experiment will hold at higher doses of nicotine. Additionally, it is unclear whether the predictive value of behaviors prior to or following early nicotine exposure will diminish with repeated drug exposures beyond 21 days. Another limitation of the current experiment is that the functions of the

mesolimbic DA system that contribute to nicotine- induced sensitization and to habituation to novelty or reduced hypoactivity were not examined. It is of interest to determine, for example, how gonadal hormones, stress hormones, and dopaminergic tone contribute to the relationships between learning and/or drug- related behaviors in both male and female rats. Animals with higher responses to novelty may exhibit higher basal DA in the NAc (Hooks et al., 1991) and/or increased corticosterone (Deroche et al., 1993; Bevins & Besheer, 2001), likely contributing to the expression of drug- induced motor activity. Further, the relationship between the response to novelty and drug sensitivity may also be mediated by central nAchR activity (Bevins & Besheer, 2001).

Certain behavioral characteristics may correlate to alterations in mesolimbic functioning may make individuals vulnerable to the effects of repeated nicotine administration. The current experiment suggests that habituation to a novel stimulus (i.e. context) can be used to predict nicotine sensitivity in females with repeated exposure and that the initial depressive effects of nicotine can be used to predict sensitivity in both males and females. Future experiments should aim to explore other early behavioral, physiological, or neural predictors of later drug sensitivity as a collection of these measures may provide characteristics by which to identify vulnerable individuals to initiate preventative measures. For example, individual differences in the rate at which nicotine is metabolized may be determined by sampling drug plasma at various timepoints following drug administration. Other stimulant effects of nicotine such as vasoconstriction, blood pressure, and enhanced cognitive alertness can be measured in humans and animals following early nicotine exposure. While difficult to measure in humans, increased basal dopamine or corticosterone can be measured in animals and likely contribute to drug

sensitivity. Early identification and understanding of the individual differences contributing to sensitivity to repeated drug effects are important in order to prevent any unnecessary exposure to addictive substances in vulnerable populations. Specifically, because women are less likely to benefit from nicotine addiction treatment (Perkins, 1999) and because existing pharmacotherapies for nicotine dependence can exacerbate mental health issues in patients with pre- existing conditions (Onor et al., 2017), it is especially important to identify which individuals may benefit most from drug abstinence.

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# APPENDIX A

# FORMULAS

***Behavioral Sensitization*** = Total activity at 21st nicotine exposure – Total activity at saline exposure

* ***Rate of Habituation*** = Total activity at 2nd exposure to chamber – Total activity at 1st exposure to chamber

* ***Hypoactivity*** = Total activity at 1st nicotine exposure – Total activity at saline exposure

***** Note: High rates of habituation and hypoactivity will be expressed as negative numbers with high absolute values whereas low rates will be expressed as negative numbers with low absolute values or positive numbers.