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CHARACTERIZATION OF SEX DIFFERENCES IN THE REINFORCING EFFECTS OF NICOTINE

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Master's Program in Experimental Psychology

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Lovingly dedicated to my beautiful wife, Monica Flores, for all the love and support she has provided me with throughout graduate school.

CHARACTERIZATION OF SEX DIFFERENCES IN THE REINFORCING EFFECTS OF NICOTINE

By

RODOLFO JESUS FLORES GARCIA, B.A.

THESIS

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Abstract

It is presently unclear whether ovarian hormones, such as estradiol (E2) promote the reinforcing effects of nicotine in females. Thus, we compared extended access to nicotine intravenous self-administration (IVSA) in intact male, intact female, and OVX female rats (Study 1) as well as OVX females that received vehicle or E2 supplementation (Study 2). The E2 supplementation procedure involved a 4-day procedure involving 2 days of vehicle administration and 2 days of E2 administration. Two doses of E2 (25 or 250 ug) were assessed in separate groups of OVX females in order to examine the dose-dependent effects of this hormone on the reinforcing effects of nicotine. The rats were given 23-hour access to nicotine IVSA using an escalating dose regimen (0.015, 0.03, and 0.06 mg/kg/0.1 ml). Each dose was self-administered for 4 days with 3 intervening days of nicotine abstinence. The results revealed that intact females displayed higher levels of nicotine intake as compared to males. Also, intact females displayed higher levels of nicotine intake versus OVX females. Lastly, our results revealed that OVX rats that received E2 supplementation displayed a dose-dependent increase in nicotine intake as compared to OVX rats that received vehicle. Together, our results suggest that the reinforcing effects of nicotine are enhanced in female rats via the presence of the ovarian hormone, E2.

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Introduction

Nicotine and tobacco use: Tobacco products contain nicotine, which has been identified as the main compound that motivates smoking behavior (Pogocki et al., 2007; Pontieri et al., 1996). For example, acute self-administration of nicotine in nonsmoking human subjects is associated with pleasurable subjective responses (Perkins et al., 2001). Similarly, nicotine increases ratings of drug high and liking in experienced smokers (Kalman et al., 2005). Humans also show a preference for nicotine versus saline in studies involving intravenous (IV; Henningfield et al., 1983; Harvey et al., 2004) and nasal (Perkins et al., 1996) administration. Taken together, these studies show that people use tobacco products largely to experience the pleasurable/reinforcing effects of nicotine

Neurobiological effects of nicotine: Nicotine exerts behavioral effects via activation of nicotinic acetylcholine receptors (nAChRs) in the brain. nAChRs belong to a family of ligand-gated ion channel receptors that are made up of five polypeptide subunits (Albuquerque et al., 1997; Lindstorm et al., 1996; Dani, 2001; Dani et al., 2001). The different combinations of these polypeptide subunits define the various nAChR subtypes (Cooper et al., 1991). nAChRs can be homo-oligomeric or hetero-oligomeric. Homo-oligomeric nAChRs are formed from α7, α8, or α9 subunits. However, hetero-oligomeric nAChRs reflect a combination of αβ, α2, α6, or β2-β4 subunits (Markou, 2008).

Previous studies have established that the reinforcing effects of nicotine are mediated via the dopaminergic pathway that projects from the ventral tegmental area (VTA) to several forebrain structures including the nucleus accumbens, amygdala, and frontal cortex (Corrigall et al., 1994; Pidoplichko et al., 1997; Sziráki et al., 2002). In the VTA, dopamine neurons are under excitatory

control via glutamate (Taber et al., 1995). Specifically, nicotine increases dopamine transmission in the nucleus accumbens by activating α7 nAChRs located on glutamatergic terminals. When nicotine activates α7 nAChRs, it causes the release of glutamate which promotes dopamine release in the nucleus accumbens. Nicotine also activates α4β2 receptors located on the terminals of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). When nicotine activates α4β2 receptors, they quickly desensitize, causing a reduction in inhibitory control of dopamine via GABA transmission. In this manner, nicotine produces an overall increase in dopamine release in the nucleus accumbens, which is believed to modulate the reinforcing effects of this drug. With regard to sex differences in nAChR expression, previous work has shown that there are no sex differences in mRNA or protein levels of various nAChRs in the nucleus accumbens (Azam et al., 2007). However, the possibility exists that chronic nicotine exposure alters nAChRs in other brain regions in a manner that enhances the reinforcing effects of nicotine in female rats.

Tobacco use in women: Clinical studies have shown that women rate nicotine as more pleasurable than men (Perkins et al., 2006). In addition, women who use tobacco regularly report higher positive subjective effects following presentation of smoking-related stimuli as compared to men (Perkins et al., 1999 & 2001). According to preclinical studies, the latter effects appear to be modulated via ovarian hormones, given that high levels of β-estradiol (E2) are positively correlated with a greater sensitivity to the reinforcing effects of nicotine in females (Carrol et al., 2004; Lynch et al., 2009). These studies suggest that women experience greater pleasurable effects from nicotine as compared to men, and that the ovarian hormones, E2, likely plays a role in promoting the reinforcing effects of nicotine.

Estrous cycle in rodents: In female rats, the menstrual cycle has three phases defined by peak fluctuations in various hormones. The three phases are the follicular, ovulatory, and luteal

phase. The proliferative phase begins at the start of menstruation and ends when ovulation occurs. During this phase, a process of follicle growth called folliculogenesis occurs. This process is mediated by the release of the pituitary hormone, follicle-stimulating hormone (FSH). Follicular growth results in the production and release of E2 from granulosa cells that surround the follicles in the ovaries. E2 then promotes the proliferation of the endometrial lining in the uterus (Beshay & Carr, 2013). Ovulation occurs during the highest expression of follicular growth because continuous E2 exposure causes the release of luteinizing hormone (LH) from the anterior pituitary, which stimulates the release of the oocyte into the fallopian tube where it remains until fertilization (Cahill et al., 1998; Pauerstein et al., 1978). After ovulation, the luteal phase begins. In this phase, the remaining granulosa cells that are not released with the oocyte during ovulation become enlarged and acquire lutein. These granulosa cells secrete progesterone, which results in the preparation of the uterus for embryo implantation (Behshay & Carr, 2013). To our knowledge the role of ovarian hormones in modulating the reinforcing effects of nicotine has not been examined in female rats.

Hormones and motivated behavior: Gonadal hormones modulate sex differences in an array of behavioral responses. For example, exposure to gonadal hormones in critical periods of development result in the sexually dimorphic organization of the nervous system in male and female rats (Becker et al., 2007 & 2009 & 2012). It is hypothesized that the sexual dimorphisms that exist in the brain are the result of the different environmental demands imposed on males and females (Yoest et al., 2014). It is also well established that gonadal hormones modulate sex differences in motivated behavior. For example, testosterone and E2 are thought to mediate sexual motivation in male (Alexander et al., 1994) and female (Paredes et al., 1999) rats, respectively. With regard to addiction processes, E2 promotes motivated behaviors in females via dopamine

systems. For example, OVX females that lack E2 display lower basal levels of extracellular dopamine as compared to intact females, and E2 treatment restores baseline dopamine levels in OVX rats (Xiao et al., 1994). Furthermore, expression of striatal D1 receptors is higher in males as compared to females and E2 downregulates D2 biding in females, but not males (Bazzett et al., 1994). The sex-dependent response of E2 in the brain is hypothesized to be due to the differential organization of neural substrates for motivation in males and females. The goal of the present study is to examine whether E2 promotes the reinforcing effects of nicotine in female rats.

Sex differences in the behavioral effects of nicotine: There are converging lines of evidence suggesting that the reinforcing effects of nicotine are greater in adult female versus male rodents (Perkins et al., 1999; Carroll et al., 2010; Torres et al., 2009; Pogun et al., 2009). One of the first studies in this area demonstrated that female adult rats display faster acquisition rates of low doses of nicotine intravenous self-administration (IVSA) as compared to males (Donny et al., 2000). The latter study also revealed that female rats reach a higher break point for nicotine infusions on a PR schedule of reinforcement than males. Subsequent studies from the same laboratory also showed that female rats display 2-fold higher levels of nicotine IVSA as compared to males in the presence of a visual stimulus that signals a nicotine infusion (Chaudhri et al., 2005) Female rats also display higher levels of nicotine intake in procedures involving oral (Nesil et al., 2011) and IV self-administration procedures under both short (Rezvani et al., 2008) and long (Grebenstein et al., 2013) access conditions. These studies suggest that the reinforcing effects of nicotine are higher in female versus male rats. Importantly, female rodents also display conditioned place preference (CPP) produced by nicotine that is evident across a wider range of doses as compared to male rats (Torres et al., 2009) and mice (Kota et al., 2008). Work in our laboratory has revealed that ovariectomized (OVX) female rats do not display CPP across an array of nicotine doses in

comparison to intact females (Torres et al., 2009). These studies suggest that the rewarding effects of nicotine are modulated via ovarian hormones.

Goals of the Master's thesis: The role of specific ovarian hormones, such as E2, in modulating the reinforcing effects of nicotine in female rats has not been explored. Thus, this Master's thesis project examined sex differences and the role of ovarian hormones in promoting the reinforcing effects of nicotine (Aim 1) and the role of E2 in modulating the reinforcing effects of nicotine in female rats (Aim 2). Nicotine IVSA was compared in intact male, intact female, and OVX female rats as well as OVX female rats that received vehicle or E2 supplementation. Two doses of E2 were included to examine the dose-dependent effects of this hormone on nicotine IVSA. An extended-access model of IVSA was employed whereby rats were given 23-hour access to increasing doses of nicotine separated by 3-day periods of drug abstinence.

Specific Aims of Master's Thesis:

Aim 1: Examine sex differences and the role ovarian hormones in modulating the reinforcing effects of nicotine.

Aim 2: Examine the role of E2 in modulating the reinforcing effects of nicotine in female rats.

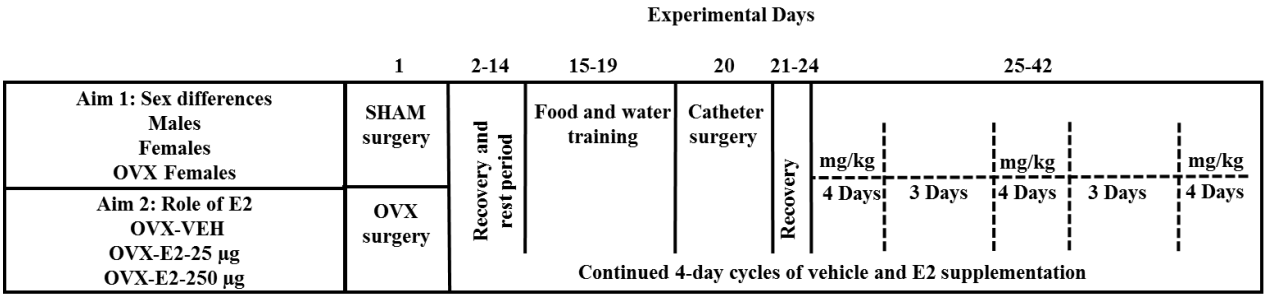
Hypotheses: Our central hypothesis is that sex differences in the behavioral effects of nicotine are ovarian-hormone dependent. Specifically, we hypothesized that: 1) females will display greater nicotine intake as compared to males and OVX females and 2) E2 supplementation in OVX rats will increase nicotine intake as compared to OVX rats that do not receive E2 replacement. Our hypothesis is based on previous studies showing that OVX females display a reduction in cocaine intake that is normalized to intact female levels following E2 supplementation (Perry et al., 2013). Also, another report revealed that OVX rats that received E2 supplementation

displayed greater motivation to obtain cocaine relative to OVX rats that received vehicle (Ramôa et al., 2013).

Methods

Subjects: Male and female Wistar rats were obtained from an out-bred stock of animals (Envigo, Inc., Indianapolis, IN). On post-natal day (PND) 21, the rat pups were weaned and housed with a same-sex littermate until PND 60, at which point they were individually housed for the remainder of the study. The rats were housed in a humidity- and temperature-controlled (22˚C) vivarium on a 12-hour light/dark cycle (lights off at 6:00 am and on at 6:00 pm). Prior to beginning the experiment, the rats were handled for 5 days and were given ad libitum access to food and water. All experimental procedures described in this Master's Thesis were approved by the UTEP Institutional Animal Care and Use Committee

Experimental design: Aim 1 compared nicotine intake in intact male, intact female, and OVX female rats. Both male and female rats received a sham surgery at PND 60 as a control procedure for the OVX surgeries. Aim 2 examined the role of E2 in modulating the reinforcing effects of nicotine in OVX females that receive vehicle (peanut oil; OVX-VEH) or an E2 supplementation procedure involving 2 different doses in separate groups of animals (E2-25 ug and E2-250 ug).



Operant procedures: This work utilized extended access procedures that are established in our laboratory (Natividad et al., 2013; O'Dell et al., 2014). IVSA was assessed in standard operant chambers (MED associates, St. Albans, VT) that were kept on the same light cycle as the holding room. Operant sessions were conducted using 2 retractable levers (active and inactive)

that extend 2.5 cm into the chamber. A 28 V white cue light was located above the active lever and a dummy light above the inactive lever. A pellet dispenser mounted between the inactive and active lever allowed the rats to nose-poke for food. A separate hole located in the back of the chamber allowed the rats to nose-poke for water that was released into an adjacent metal dipper cup. The exit port of the catheter fitting was connected to a polyethylene tubing within a metal spring that was connected to a liquid swivel above the operant chamber. During the first 4 days of operant procedures, the rats received food and water training. The rats were allowed to nose-poke for the delivery of food pellets (45 mg; Bio-Serv; Frenchtown, NJ) or water (0.1 mL) on a fixed-ratio 1 (FR-1) schedule of reinforcement. Throughout the operant procedures, the rats were removed from the chambers between 11:00 am and 12:00 pm in order to clean the cages and replenish the water and food levels. Immediately after being removed from the chambers, the rats were weighed and placed individually into their home cage. On the first day of IVSA, the rats were presented with a novel active and inactive lever at 12:00 pm. The rats were given access to various doses of nicotine IVSA on an FR-1 schedule of reinforcement using an escalating dose regimen of nicotine (0.015, 0.03, and 0.06 mg/kg/0.1 mL infusion; base). When the active lever was pressed, the nicotine solution was delivered at a rate of 0.1 mL per second. At the onset of the 1-second infusion, a cue light was illuminated above the lever for 20 seconds. This was followed by a 20-second time out period. Responses on the inactive lever did not have any scheduled consequences. The nicotine solutions were prepared daily based on the animals' weight from the previous day. A nicotine stock solution was prepared for each IVSA dose using (-) nicotine hydrogen tartrate (Sigma-Aldrich, St. Louis, MO) dissolved in 0.9% sterile saline (pH of 7.4). Each dose of nicotine was administered in a 4-day cycle with 3 intervening days of drug abstinence. During the 3-day abstinence period, the rats were housed in their home cage with ad libitum food and water.

Surgical procedures: At PND 45-46 some female rats received surgical removal of ovarian tissue, as described previously (Torres et al., 2009). The OVX procedure was done at PND 45-46 based on previous work in our laboratory showing that adult female rats that received OVX procedures at PND 45 display a reduction in the reinforcing effects of nicotine (Torres et al., 2009) and a suppression of anxiety-like behavior and stress-associated gene expression during nicotine withdrawal (Torres et al., 2013 & 2015). These studies suggest that after PND 45 ovarian hormones play a key role in modulating the behavioral effects and molecular changes produced by nicotine.

At PND 65, the rats were anesthetized with an isoflurane/oxygen vapor mixture (1-3%) and prepared with jugular catheters, as described previously (Natividad et al., 2013; O'Dell et al., 2014). Following surgery, the rats were allowed to recover for four days and the catheters were flushed daily with a 0.2 mL infusion of an antibiotic solution containing Timentin (100 mg/ml) and heparinized saline (30 USP units/mL). Prior to nicotine IVSA, the catheter patency was verified using a 0.1 mL IV infusion of the short-acting barbiturate Brevital® sodium (10 mg/mL). Patency tests were also conducted when aberrant shifts in behavior are detected, and non-patent animals will be excluded from the study.

E2 supplementation procedure: The rats in Aim 2 received a 4-day E2 supplementation procedure that began the day after the OVX surgery. Control OVX females received repeated vehicle injections (peanut oil). OVX females that received the E2 supplementation procedure received 2 days of a 0.2 ml bolus injection of E2 (25 or 250 ug) and 2 days of vehicle injections. The E2 supplementation procedure was repeated 4 times, prior to and throughout IVSA testing. The injections were administered each day between 11:00 am and 12:00 pm when the animals were removed from the operant chambers. This supplementation procedure is believed to mimic normal E2 cycling patterns in intact female rats (Asarian et al., 2002). The latter study was also

used to guide our selection of a low physiological dose of E2 (25 ug) and a significantly higher dose (250 ug) that is expected to produce strong pharmacological effects.

Statistics: Average nicotine intake was calculated on a daily basis across different doses of nicotine. Each study was analyzed separately using a mixed-measures analysis of variance (ANOVA) with group as the between-subjects factor and dose and day within-subjects factors. Where significant interactions were observed, post-hoc (Fisher's LSD) comparisons were conducted between groups.

Results

Study 1

Figure 1 depicts nicotine IVSA (mg/kg) in intact male, intact female, and OVX female rats. The panel on the left reflects daily intake, and the panel on the right reflects mean intake of each dose. Overall, the results revealed that female rats display dose-dependently higher levels of nicotine intake as compared to intact males and OVX females. Our analysis of daily intake in the left panel revealed a 3-way interaction between group, dose, and day ($F_{(12,180)}$=2.2, P≤0.01). Specifically, intact females display higher levels of nicotine intake as compared to both intact males and OVX females on Days 5-6 and 11-12 (*P≤0.05). Also, intact females display higher levels of nicotine intake as compared to OVX females on Day 7, 8, and 10 (†P≤0.05). Our analysis of mean intake in the right panel revealed a 2-way interaction between dose and day ($F_{(4,60)}$=3.7, P≤0.01). Intact females display higher levels of nicotine intake as compared to both intact males and OVX females at the 0.06 mg/kg dose of nicotine (*P≤0.05). Also, intact females display higher levels of nicotine intake as compared to OVX females at the 0. 015 and 0.03 mg/kg dose of nicotine (†P≤0.05). Our group differences in Study 1 do not appear to be related to inactive lever pressing, since there were no differences in mean total responses on the inactive lever across IVSA days in intact male (17.9±4.9), intact female (24.9±5.7), and OVX female (27.5±4.7) rats ($F_{(2,40)}$=1.0, P=ns).

Study 2

Figure 2 depicts nicotine IVSA (mg/kg) in OVX female rats that received vehicle or E2 supplementation. The panel on the left reflects daily intake, and the panel on the right reflects mean intake of each dose. Overall, the results revealed that OVX female rats that received the high dose of E2 display greater nicotine intake as compared to OVX females that received vehicle and the

low dose of E2. Our analysis of daily intake in the left panel revealed a 3-way interaction between group, dose, and day ($F_{(12,138)}$=2.2, P≤0.01). Specifically, OVX female rats that received the high dose of E2 display greater nicotine intake as compared to OVX female that received vehicle on Day 1-6, 8, and 10-11 (*P≤0.05). Also, OVX female rats that received the low dose of E2 displayed greater nicotine intake as compared to vehicle controls on Day 3 (*P≤0.05). With regard to dose-dependent effects of E2, OVX female rats that received the high dose of E2 displayed higher levels of nicotine intake as compared to rats that received the low dose of this hormone on Day 4, 7, and 9-10 (†P≤0.05). Our analysis of mean intake in the right panel revealed that OVX female rats that received the high dose of E2 displayed greater nicotine intake as compared to vehicle controls at each dose of nicotine (*P≤0.05). Also, OVX females that received the low dose of E2 displayed greater nicotine intake as compared to vehicle controls at the 0. 015 mg/kg dose of nicotine (†P≤0.05). With regard to dose-dependent effects of E2, OVX female rats that received the high dose of E2 displayed greater nicotine intake as compared to rats that received the low dose of E2 at the 0.03 mg/kg dose of nicotine (†P≤0.05). Our group differences do not appear to be related to disparities in inactive lever pressing, since there were no differences in mean total responses on the inactive lever across IVSA days in OVX female rats that received vehicle (30.8±7.4), E2 25 ug (25.6±5.8), and E2 250 ug (38.8±9.5) administration ($F_{(2,46)}$=0.7, P=ns).

Discussion

In summary, my thesis work revealed that the reinforcing effects of nicotine are greater in intact female versus male rats. The latter effect appears to be hormone dependent, as the strong reinforcing effects of nicotine observed in intact females are reduced in female rats lacking ovaries. The unique contribution of this work is that E2 supplementation increases nicotine intake in OVX females as compared vehicle controls.

Our finding that intact females display greater reinforcing effects of nicotine than males is consistent with previous reports. In fact, there are now several reports showing that adult female rats display greater rewarding effects of nicotine across an array of IVSA (Donny et al., 200;Chaudhri et al., 2005; Nesil et al., 2011; Rezvani et al., 2008; Grebenstein et al., 2013) and CPP (Torres et al., 2009; Kota et al., 2008) experimental conditions. However, we acknowledge other reports showing that adult female rats display similar (Feltenstein et al., 2012) or lower (Johnson et al., 2012) levels of nicotine intake as compared to males. The notion that the reinforcing effects of nicotine are greater in females is also supported in previous studies that compared sex differences in nicotine intake during the adolescent period of development. For example, adolescent female rats acquired nicotine IVSA at lower doses (Lynch et al., 2009) and display higher levels of nicotine intake under extended access conditions (Sanchez et al., 2014) as compared to males. Another series of studies revealed that female rats that initiated nicotine IVSA during adolescence display an escalation of nicotine intake into adulthood, but this effect was not observed in males (Levin et al., 2003 & 2007). The present study contributes to a large body of literature suggesting that adult female rats are more sensitive to the rewarding effects of nicotine than males.

One possibility to consider is that behavioral sensitization may have contributed to the greater nicotine self-administration in female versus male rats. Behavioral sensitization is observed as an increase in behavioral effects following repeated exposure to a psychostimulant drug, such as nicotine (Vanderschuren & Kalivas, 2000). Thus, one important consideration is that chronic nicotine exposure produced an enhancement of stimulant behavior in female rats that may have lead to an increase in lever pressing behavior in females. Indeed, one study found that female rats display greater locomotor and rearing behavior as compared to males following 21 days of nicotine exposure (Harrod et al., 2004). Another study found that female rats displayed more locomotor activity as compared to males following continuous nicotine exposure in osmotic pumps (Cronan et al., 1985). Importantly, another study also found that females displayed greater nicotine-induced sensitization after 14 daily IV infusions of nicotine as compared to males, an effect that was reduced in OVX female rats (Booze et al., 1999). Although the development of behavioral sensitization following chronic nicotine self-administration may have contributed to the enhanced lever pressing for nicotine in females, there were no sex differences in responding on the inactive lever. These data suggest that the female rats were selectively pressing the nicotine lever because of strong reinforcing effects of this drug, and not greater stimulant effects of nicotine as compared to males.

Our finding that OVX rats display reduced nicotine IVSA as compared to intact females suggests that ovarian hormones mediate the reinforcing effects of nicotine in female rats. This is consistent with previous findings in our laboratory showing that OVX rats do not display CPP across an array of nicotine doses (Torres et al., 2009). Similarly, OVX female rats do not display CPP produced by ethanol (Torres et al., 2014) and they acquire IVSA of cocaine (Hu et al., 2004; Lynch et al., 2001) and heroin (Roth et al., 2001) at slower rates than intact females. These findings

suggest that the reinforcing effects of drugs of abuse are modulated by the presence of ovarian hormones.

The unique contribution of the present study is that E2 supplementation dose-dependently increased the reinforcing effects of nicotine in female rats lacking ovarian hormones. The finding that the strong reinforcing effects of nicotine are normalized in OVX rats that receive E2 supplementation suggests that E2 is an ovarian hormone that modulates the reinforcing effects of nicotine. E2 has been identified as an ovarian hormone that modulates the reinforcing effects of drugs of abuse, such as cocaine (Becker et al., 2012). Indeed, previous reports have revealed that OVX rats that received E2 supplementation acquired cocaine (Hu et al., 2004; Lynch et al., 2001; Jackson et al., 2006) and heroin (Roth et al., 2002) IVSA more readily as compared to OVX rats that received vehicle. Our findings extend the literature by showing that E2 also modulates the reinforcing effects of nicotine.

It has been suggested that E2 promotes nicotine reward via an enhancement of dopamine transmission in the mesolimbic pathway, which originates in the ventral tegmental area and terminates in several forebrain structures including the striatum and nucleus accumbens (Becker et al., 2008; Dluzen et al., 1997; Van Vorrhees et al., 2012). OVX female rats display a reduction in synaptic levels of dopamine in the striatum that is normalized by following E2 supplementation (Ohtani et al., 2001). Also, acute administration of E2 enhances dopamine release via activation E2 receptors in the striatum, in female but not male rats (Castner et al., 1993; Becker et al., 1990). It has been posited that E2 receptors in the NAcc are located on the terminals of inhibitory gamma-aminobutyric acid (GABA) medium spiny neurons, such that activation of E2 receptors disinhibits GABA and increases dopamine release in the striatum (Yoest et el., 2014). Thus, it is possible that E2 promotes the reinforcing effects of nicotine via an increase in dopamine transmission. We also

recognize the importance of other ovarian hormones, such as progesterone that has been shown to play a role in modulating drug use in females (Carrol et al., 2010; Lynch et al., 2010). Indeed, a previous study revealed that peak plasma levels of progesterone are negatively correlated with nicotine IVSA in adolescent female rats (Lynch et al., 2009). Future studies are needed to examine the intricate relationship between E2 and progesterone in modulating the reinforcing effects of nicotine in females.

The present findings provide several clinical implications to consider. First, our finding that the reinforcing effects of nicotine modulated via ovarian hormones suggests that women smokers may display compromised smoking cessation rates following various types of hormone therapies, such as the E2 antagonist Tamoxifen and/or birth control methods. Indeed, pre-clinical studies have revealed that Tamoxifen increases conditioned place preference produced by nicotine in female rats (Yararbas & Pogun, 2011). Second, the finding that E2 promotes the reinforcing effects of nicotine suggests that E2 may play a central role in promoting tobacco use in women. Indeed, clinical studies have shown that women in the follicular phase of the menstrual cycle report greater positive subjective effects of nicotine as compared to phases of the cycle (Devito et al., 2013). Thus, it is possible that high levels of E2 promote nicotine use and relapse. Future work is needed to determine whether E2 promotes the reinforcing effects of nicotine. Future studies might also examine the role of other ovarian hormones, such as progesterone in tobacco use. Indeed, progesterone treatment has been shown to enhance the subjective ratings of the negative effects of nicotine and attenuate the pleasurable effects of this drug following IV nicotine administration (Sofuoglu et al., 2009). Future studies are warranted to examine the complex relationship between ovarian hormones and tobacco use in women.

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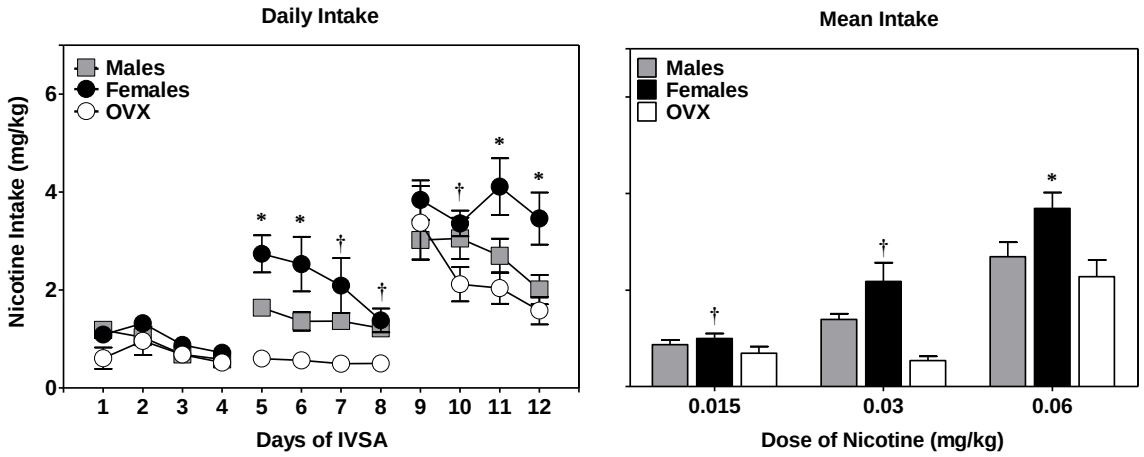

Figure 1: This figure depicts nicotine IVSA depicted as daily intake (left panel) and mean intake (right panel) in intact male (n=10), intact female (n=14), and OVX female (n=9) rats from Study 1. Rats were given 23-hour access to escalating doses of nicotine (0.015, 0.03 and 0.06 mg/kg) for 4 consecutive days separated by 3 days of drug abstinence. Intact female rats displayed higher levels of nicotine intake as compared to males and OVX females. The asterisks (*) denote significantly higher intake in intact females as compared to both intact males and OVX females, and the daggers (†) denote higher intake in intact females as compared to OVX females ($P<0.05$).

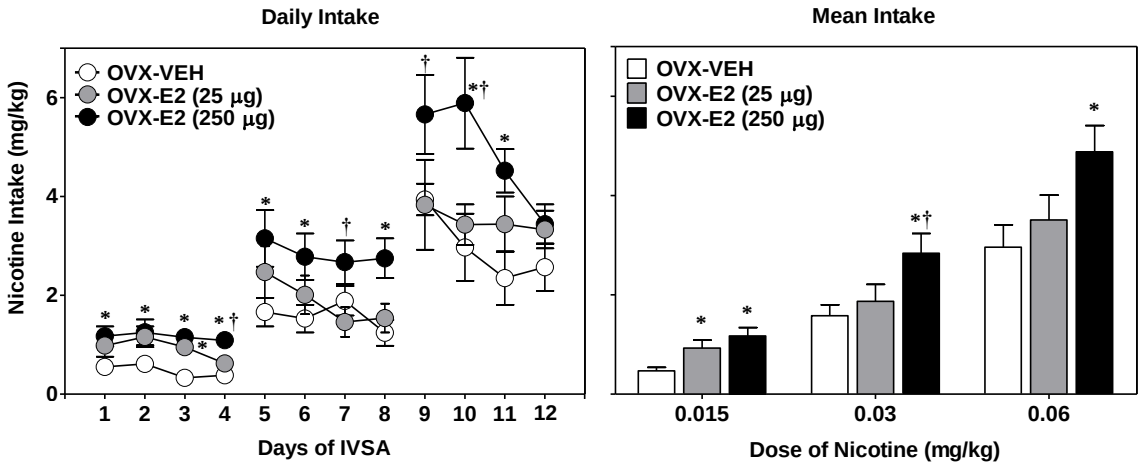

Figure 2: This figure depicts nicotine IVSA depicted as daily intake (left panel) and mean intake (right panel) in OVX female rats that received vehicle (OVX-VEH; n=8) or E2 supplementation at a low (OVX-E2 25 ug; n=8) or high (OVX-E2 250 ug; n=10) dose of this hormone in Study 2. OVX females that received the high dose of E2 displayed higher levels of nicotine intake as compared vehicle controls and OVX females that received the low dose of E2. The asterisks (*) denote significantly higher intake in OVX females that received E2 as compared to vehicle controls, and the daggers (†) denote higher levels of intake in OVX females that received the high versus low dose of E2 ($P<0.05$).

Curriculum Vitae

Rodolfo J. Flores Garcia was born to Rodolfo Flores and Martha Garcia in Naucalpan, Estado de México, México. He graduated from San Pedro High School in San Pedro, California in May 2008 and entered California State University, Long Beach (CSULB) in Long Beach, California, the following semester. During his undergraduate career, Rodolfo was a tutor in the Boys and Girls Club of America where he mentored underprivileged students from diverse backgrounds. He became interested in Neuroscience and began research work with Dr. Arturo R. Zavala who studied the effects of early exposure to methylphenidate in the rewarding effects of methamphetamine during adolescence in rats. He became interested in investigating the factors that make individuals more vulnerable to become addicted to drugs. Therefore, in the summer of 2013, he joined the laboratory of Dr. Laura E. O'Dell at the University of Texas at El Paso (UTEP) as part of a summer research internship. Her laboratory combines behavioral, molecular and biochemical techniques to study the factors that promote tobacco use in vulnerable populations. During the internship, he primarily worked in a research project that investigated the role of insulin in the reinforcing effects of nicotine in an animal model of diabetes. He obtained his Bachelor of Arts degree in Psychology with a minor in Chemistry from CSULB in May 2014. He entered the Social, Cognition, and Neuroscience program at UTEP in August, 2014 where he received mentorship from Dr. Laura E. O'Dell to investigate the factors that promote tobacco use in females. Rodolfo has presented his research work in 25 conferences focused on drug abuse. He is first-author on 1 published article in *Behavioural Brain Research* and co-author in 1 published article in *Pyschopharmacology*.

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