Effects of Central vs. Peripheral Estrogen Activity on Nicotine-Induced Conditioned Place Preference in Female Rats



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Abstract

Nicotine dependency is an ongoing problem in the United States, and with modern forms of nicotine intake, such as vaporizers, is affecting younger people every year. It is believed that females are especially susceptible to the adverse effects of nicotine including addiction onset, problems with cessation, withdrawal, and mortality. These effects are believed to be caused by estrogen; a hormone produced by the ovaries. Estrogen is believed to directly effect GABA function in the nucleus accumbens and striatum, resulting in enhanced dopamine activity. Using female rats in three conditioning groups, controls, ovariectomized (OVX), a group receiving a peripheral anti-estrogen called ICI 182,780 (ICI) (n=6 per group, n=18 total), the present study aimed to determine if estrogens effects on female addiction were due solely to central nervous system input or if the peripheral nervous system also plays a role. A biased conditioned place preference (CPP) paradigm was used with animals receiving s.c. injections of nicotine at a dose of 1.2mg/kg. Results indicated that controls and ICI's displayed higher CPP scores than OVX animals. However, differences in CPP scores from controls and ICI's to OVX's did not differ greatly, explained possibly by small sample sizes.

Keywords: Nicotine, Conditioned Place Preference, Estrogen, OVX, ICI 182,780

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Introduction

Nicotine is an extremely addictive substance that comes from tobacco. Tobacco use is the leading cause of preventable death in the US and is known to have serious negative effects on the mind and body such as addiction, cancer, heart disease, stroke, lung diseases, diabetes, and chronic obstructive pulmonary disease, which includes emphysema and chronic bronchitis (CDC, 2019a). However, while consuming tobacco itself causes health concerns, the addiction comes from nicotine. This is important because while smoking tobacco in America has decreased, the use of nicotine is becoming popular in the form of electronic nicotine devices (END) (CDC, 2019b; NCCPHP, 2014; NIDA, 2020; Lora, 2018), specifically with adolescents and young adults (1 in every 20 middle school students and 1 in every 5 high school students) (CDC, 2019b). From 2017 to 2018 the use of nicotine via ENDs increased by 38.3% in middle school and high school students (Loria, 2019). While the effects of ENDs on health are still being studied, researching the behavioral and physiological effects of nicotine could help give us a better understanding of how it contributes to addiction regardless of its vehicle. 12.2% of women are current smokers as of 2017 (CDC, 2019c). Numerous research review focusing on general nicotine addiction and/or the effects of sex on the epidemiology of nicotine (Allen et al., 2014a; Benowitz, 2010; Cross et al., 2017; Wetherill et al., 2016) as well as meta-analytic studies using the menstrual cycle to measure subjective effects of nicotine (Allen et al., 2014b; Weinberger et al., 2015) have concluded that females are more susceptible to the adverse effects of nicotine including addictive potential, withdrawal, cessation, and mortality. ENDs pose a new danger to women because of the large amounts of nicotine they contain and the chance that they can turn to using tobacco products in the future. The sex differences in nicotine addiction have been linked to gonadal hormones, specifically estradiol, a hormone produced by the ovaries.

Nicotine acts on a specific class of ligand-gated ion channels called nicotinic acetylcholine receptors (nACHrs). These receptors are excitatory and usually bind acetylcholine, the most common type of these being the α4β2 receptor. When bound to nicotine, these receptors will allow sodium or calcium into the cell, which depolarize the cell causing more calcium influx from voltage-dependent calcium channels (Benowitz, 2010). Higher amounts of calcium in the cell effects its function in two ways. 1. In the cell body, higher calcium influx raises the resting membrane potential closer to minimum levels for an action potential to be generated. 2. It causes an increase in neurotransmitter release by allowing vesicles within the axon terminal to fuse to the membrane and be released into the synaptic cleft. Nicotine triggers the release of dopamine into the mesolimbic area, striatum, and frontal cortex. Nicotine also changes the function of glutamate release and, with long-term exposure, decreases GABA-mediated inhibition, which enhances excitation of dopaminergic neurons and enhances the response to nicotine (Benowitz, 2010).

One study using *Xenopus laevis* ooctyes found that estradiol may have a specific binding site on α4β2 nicotinic acetylcholine receptors (nAChRs), causing increases in receptor affinity and allosteric potentiation of the receptor's responses (Curtis et al. 2001). Studies using rats show that Estradiol decreases GABA release in the striatum by inhibiting calcium channel current in medium spiny GABAergic neurons (HU, Watson, Kennedy, and Becker, 2005). Previous studies with rats have shown that subcutaneous injections of estradiol, measured by in vivo microdialysis, greatly enhances DA release in the striatum (Becker and Rudick, 1999), while estrogen treatments do not have the same effect on male rats (Becker, 1999). Figure 1 illustrates a proposed method of how estrogen acts to enhance dopamine release in female rats. Interestingly, while estradiol is thought to enhance vulnerability to continued smoking and

relapse, progesterone is thought to be protective against these effects (Lynch and Sufuoglu, 2010), although physiological levels of progesterone may not be high enough to counteract the effects of estradiol.

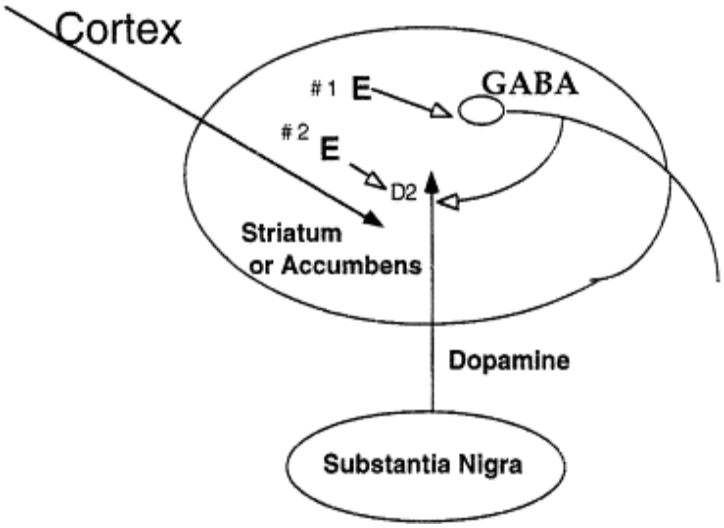


Fig. 1. Two mechanisms are postulated to contribute to the effect of E on stimulated DA release in female rats: #1-Estrogen acts to inhibit intrinsic GABA neurons that have recurrent collaterals onto DA terminals. This results in higher DA release. #2- Estrogen acts on DA terminals to enhance DA release by downregulating presynaptic D2 DA receptor function (figure and legend from; Becker pg.806, 1999).

Research on animals suggests that females develop nicotine related addictive behaviors (conditioned place preference, intravenous self-administration, locomotor activity) at lower doses compared to males (Torres et al. 2009). Females will also exhibit higher nicotine plasma levels compared to males when IV injected with nicotine (Harrod et al. 2006), however studies suggest that any nicotine related behaviors can be attenuated by ovariectomy in females (Torres et al. 2009; Harrod, Booze and Mactutus. 2006). Conditioned-place preference (CPP) is a commonly used model for testing addiction in animals. Numerous studies have shown successful conditioning of male, female, and adolescent rats using nicotine at a range of doses (0.01-1.4mg/kg) (Harrod et al. 2006; Natarajan et al. 2011; Brielmaier et al. 2007; Ahsan et al. 2014; Belluzzi et al. 2004; Le Foll and Goldberg, 2004; Torres et al. 2009; Matta et al. 2007). Most studies using CPP and nicotine do not look at sex-differences, however, studies that do, find that

female rats will have a greater response to nicotine at the same doses as males (Torres et al. 2009) and will have higher plasma nicotine levels compared to males at the same doses (Harrod 2006). These studies also found that both sexes of rats will develop conditioned-place aversion (CPA) at higher doses of nicotine, and adolescent rats will develop CPP or CPA at lower doses compared to adults (Torres et al. 2009; Beluzzi et al. 2004; Ahsan et al. 2014; Brielmaier et al. 2007; Natarajan et al. 2011). The aim of this study was to test whether the effects of estrogen found in other studies require peripheral action, or if the effects are purely central by using a drug called ICI 182,780 (a "pure" peripheral estrogen blocker, void of any agonistic effects). Animals receiving this drug will be labeled as 'ICI'. This drug has been tested successfully for peripheral but not central estrogen blocking in pre-clinical and clinical trials in humans (Howell et al. 2000), in mice (Andersson et al. 2005), and in rats (Rivera and Eckel, 2010). In addition to this, I plan to replicate previous findings on the addictive potential to nicotine found between intact females and OVX females given the same dose.

The present study looked at the effects of central and peripheral nervous system estrogen activity on nicotine-induced CPP in female rats. With 3 distinct conditioning groups, I hoped to provide evidence for the theory that heightened sensitivity to nicotine in female rats was due not only to estrogen produced by the ovaries, but also distinctively from input of the central nervous system. I hypothesize that in all measures of CPP (not including pretest scores alone), controls and ICI's will have similar results and will both have scores higher than the OVX's. I also hypothesize that uterine (UT) weight will be similar for the ICI's and OVX's, while the controls will have higher UT weights.

Methods

Subjects:

Adult female Sprague-Dawley rats, were used for the experiment. Conditioning groups included intact females (n=6), ICI females (n=6) and OVX females (n=6). Rats were bred at Allegheny College and were housed in pairs in plastic cages. Rats had regular access to food and water throughout the study and were weighed every day. All rats were kept on a 15/9-hour light/dark cycle and were handled 2-3 min for 2-4 days before experimentation.

Drugs:

(-)-Nicotine hydrogen tartrate was obtained from Sigma Aldrich. Nicotine was diluted in saline with a PH adjusted to 7.0 with dilute NaOH. Nicotine was administered in a volume of 1.0 ml/kg at a dose of 1.2 mg/kg to each animal (Torres et al. 2009). Saline was administered in a volume of 1.0ml/kg. Dosages for nicotine are expressed as mg free base per kg body weight. The formula (dose x BWt x (MW of salt nicotine/ MW of nicotine free base) was used to convert salt dosages to free base dosages (MW of salt nicotine= 498 g/mol, MW of nicotine free base= 162 g/mol) (Matta et al. 2007). ICI 182,780 was purchased from Sigma Aldrich. It was administered at a dose of 0.5mg/kg in 1ml/kg in saline (0.04% alcohol) (Rivera and Eckel 2010) and was administered in a volume of 1.0ml/kg.

Conditioned place preference protocol:

The CPP apparatus consisted of a clear plexiglass box 36(L) x 12(W) x 12(H)in with two conditioning compartments (12 x 12 in) separated by a neutral compartment (12 x 12 in). The two outside chambers had either a rubber floor with a ribbed, or a rubber floor with a smooth texture. A piece of cardboard 38(L) x 14(W) x 14(H)in was placed over the conditioning apparatus during all phases of conditioning. The cardboard surrounding the apparatus was meant

to control for any visual stimuli in the room. Animals were assessed for their preference using a biased design in which animals were assigned to their initially non-preferred chambers following nicotine administration, and to their initially preferred room when injected with saline. A stopwatch was used to record times spent in chambers. The number of times a rat enters each chamber were also recorded. The rats were considered to be in a chamber if both front paws were in the compartment. This conditioning protocol was based on a study by Sun et al., 2018, and modified due to time constraints and smaller sample sizes.

Pre-condition:

One day of pre-conditioning was performed in each rat. Rats were placed in the center chamber of the CPP box with access to all three chambers for 15 minutes. This tested each rat's initial preference for each compartment.

Conditioning:

At least 1-2 days after the pre-conditioning phase, rats spent 6 days being conditioned to their non-preferred chamber. Rats were randomly assigned to receive an injection of nicotine or saline on day 1 of the conditioning phase. Rats in both groups then received either an injection of nicotine or saline once a day for the rest of the conditioning period. ICI animals received injections of ICI 182,780 once a day beginning one day before the pretest with the last injection on the final day of conditioning. The drug was given approximately an hour to be metabolized by animals before receiving an additional injection of nicotine or saline. When rats were injected with nicotine, they were placed in their initially non-preferred chamber for 20 minutes. Rats were placed in their initially preferred chambered for 20 minutes when injected with saline. During conditioning, the doors between the compartments were closed so that the rats did not have

access to other compartments.



Fig 2. Timeline for CPP conditioning and testing. Green bars indicate a day with nicotine injection, the red bars indicate a day with saline injection. Blue bars represent the pretest and posttest that were performed drug-free. Because a counter balanced order was used, colored bars would be revered for animals that received injections of saline on the first conditioning day. First day injections were randomly assigned within each conditioning group. The first 2 weeks of experimentation ran 3 animals per cycle. The remaining weeks ran 6 animals at a time, n=3 from the ICI group and n=3 from the OVX group for 2 weeks. ICI and OVX animals were mixed during the last 2 weeks of condition due to the different recovery rates of OVX animals from surgery.

Post Testing:

Posttests were conducted 1-2 days after the condition phase due to time constraints and the amount of animals being conditioned at a time (e.g., n=3 at a time for the first two weeks and n=6 at a time for the remaining weeks). Rats were placed in the center chamber of the CPP box drug free and allowed full access to all three chambers. Rats preference for each compartment was observed for 15 minutes.

Surgery:

All Surgical supplies were acquired through Allegheny College department of Neuroscience. All animals had at least 5 days of recovery time before experimental manipulations were made. Surgery animals were placed in isolated cages for the remainder of the experiment until sacrificed. Antibiotics mixed with peanut butter were placed in their cages once a day for 3 days following surgery (some received these antibiotics every day and lidocaine some days around their stiches if the incision looked irritated and was unable to be reclosed). Weight, behavior, and pain were monitored daily for all surgical animals during the entirety of the study.

Ovariectomy:

Female rats were anesthetized with an i.p. injection of Ketamine and Xylazine, mixed at [70mgKet+6mgXyl/ml]. Dosages were 0.1cc/100g BWt. Both ovaries were removed with a 2CM incision made rostral to the crest of the ilium of the pelvic girdle and posterior the ribcage on both sides. The area of the skin was shaved and swabbed with Betadine and then alcohol. Once through the skin, further incisions were made through the musculoperitoneal wall, and then a pad of adipose tissue. The adipose pad, ovary, and uterine horn were all exteriorized before a ligature was tied around the uterine horn. With the ligature secured, it was tightened, cutting off blood to the distal tissues. The distal tissue was then cut off just above the formed ligature, which included the ovary, ovarian duct and some of the fat pad. The remaining exteriorized tissue was carefully placed back into the cavity before being sutured. Animals were given 5 days for recovery between surgery and experimentation.

Uterotrophic bioassay:

Approximately 2-4 days after the posttest, rats were euthanized with Somnasol and perfused with formaldehyde. After the head was removed, the body cavity was opened further to allow access

to the uterus. The uterus was excised at the base of the cervix (for animals not in the OVX group, the uterine horns were cut approximately 2-4mm below the ovary and adipose fat pad to compensate for what was removed during the ovariectomy surgery).

Data analysis:

CPP scores were calculated in percent ratios of time spent in each of the conditioning compartments during the pretest and posttest of condition. The calculation was as follows: time spent in non-preferred chamber(s) / time spent in both chambers(s). The time spent in the center compartment was subtracted so that the total times would equal 100%. Pretest and posttest CPP scores were analyzed with one-way ANOVA.

UT weight was also analyzed with one-way ANOVA. The uterotrophic bioassay was used as an indicator of peripheral estrogen activity and has been validated by the Organisation for Economic Co-operation and Development (OECD) in an international validation study (Kanno et al., 2001). Lower uterine (UT) weights indicated lower peripheral estrogen activity.

Pre vs posttest body weight, pretest vs posttest CPP scores, and pretest vs posttest total entrances were analyzed with two-way mixed-model ANOVA's. Total entrances into the chambers during the pretest and posttest were a measure of increase in locomotor activity. Apparatus bias was calculated by taking the total time(s) spent by animals in each conditioning chamber during the pretest and were totaled across all 3 conditioning groups. This was analyzed with a paired-sample *t* test. Post-hoc tests were performed for all ANOVA's where appropriate.

Results

Animal body weight from pretest to posttest:

Pre-weights were taken the same day as each rat's pre-test and post-weights were taken on the same day as each rat's post-test. The results of a Two-Way Mixed ANOVA indicated a main effect of weight ($F(1,15)=15.954$, $p=.001$, $\eta p2=.515$). As shown in Figure 3, controls and OVX rats increased in weight from the pretest to post-test, while ICI rats did not. There was a main effect of the condition groups, ($F(2,15)=5.256$, $p=.019$, $\eta p2=.412$). There was also an interaction of weight X condition group ($F(2,15)=8.127$, $p=.004$, $\eta p2=.520$). A Tukey post-hoc analysis revealed that OVX rats increased in weight from the pretest to posttest (M_{pre}=268.5g, SD=19.63; M_{post}=308.6g, SD=15.74) more than controls (M_{pre}=250.2g, SD=16.18; M_{post}=263.4g, SD=18.27, $p=.015$).

Expression of nicotine-induced CPP:

CPP scores were calculated for the pretest and the posttest of the conditioning phase. CPP post-test scores were calculated to determine the strength of preference, expressed as a ratio of time spent in their non-preferred chamber to the total time spent in both chambers. The lower the CPP score indicates less time spent in their non-preferred chamber. A one-way ANOVA indicated that CPP post-test scores varied by condition group (Figure.4), $F(2,17)=4.41$, $p=.031$. A Tukey post-hoc test revealed that ICI rats had stronger preference for their non-preferred chamber (M=0.588, SD=0.0636) following the conditioning phase than OVX rats (M=0.471, SD=0.0567, $p=.034$). Differences between controls and ICI's were non-significant, $p>.05$, and though controls had higher CPP scores than OVX's, this difference was also non-significant, $p=.094$.

The results of a Two-Way Mixed ANOVA showed that there was a main effect of the CPP scores, ($F(1,15)=29.514$, $p>.001$, $\eta p2=.663$), on the conditional groups, ($M\text{-}pre_{total}=.392$, $SD=.099$; $M\text{-}post_{total}=.542$, $SD=.085$) (Figure.5). There was no main effect of conditioning group, ($F(2,15)=2.521$, $p=.114$, $\eta p2=.252$). Because the main effect of conditioning groups was $p=.114$, a Tukey post-hoc test was performed to determine where the differences could have been significant. Post-hoc analysis revealed that the mean difference in scores for controls and OVX's was 0.075, $p=.156$, and that the difference in scores for ICI's and OVX's was 0.074, $p=.166$. The mean difference in scores between controls and ICI's were 0.0014, $p=.999$.

Apparatus bias:

A paired-samples t-test was conducted to test for the existence of any bias of the stimuli chosen for the conditioning apparatus by comparing the time(s) spent in each chamber during initial preference testing. There was no significant difference in the time spent in the ribbed mat compartment ($M=243.22$s, $SD=90.977$) and the smooth mat ($M=198.50$s, $SD=87.499$) compartment; $t(17)=1.487$, $p=.150$, 95% *CI* [-16.130, 91.575] even though 12 rats initially preferred the ribbed mat while 6 preferred the smooth mat (Figure 6). Furthermore, CPP pre-test scores were calculated to determine if the proportion of time rats spent in their preferred chamber during the initial preference test varied significantly. A one-way ANOVA indicated that the CPP pre-test scores did not vary significantly between conditioning groups, $F(2,17)=.471$, $p=.633$ (Figure 7).

Uterine weight:

A one-way ANOVA indicated that uterine weight did not vary significantly between conditioning groups, $F(2,17)=2.487$, $p=.117$. A Tukey post-hoc test did indicated a mean difference between the control rats (M=504mg, SD=143.6mg) and OVX rats (M=273.3mg, SD=229.8mg) of 230.6mg, $p=.117$ (Figure 8).

Locomotor activity:

A Two-Way Mixed ANOVA showed that there was a main effect of entrances into the chambers ($F(1,15)=21.619$, $p<.001$, $\eta p2=.590$). There was no main effect of conditioning group ($p>.05$). as shown in Figure 9, across all conditioning groups there was an increase of total entrances into the chambers from the pre-test ($M\text{-}ent_{total}$=23.5, SD=9.03) to the post-test ($M\text{-}ent_{total}$=44.06, SD=19.5).

Discussion

The present study explored the effects of estrogen on nicotine-induced addictive behavior in female rats as well as whether such effects were driven by peripheral nervous system or central nervous system activity. Using a biased CPP procedure, tactile stimuli were chosen for the conditioning chambers, similar to the work of Le Foll and Golberg, 2005. Like Le Foll and Goldberg, using tactile stimuli for the conditioning apparatus resulted in weak initial bias. This is contrasted by other studies which used visual stimuli for their conditioning apparati, having one chamber white and the other black (e.g., Brielmaier et al., 2007 and Natarajan et al., 2011). These studies resulted in a very strong initial bias for the black chamber. Natarajan et al. also

found that when a lamp was applied to black chamber, the bias evened out. The present study found that while 2/3 of the animals preferred the ribbed mat over the smooth mat, this was not statistically significant. Figure 4 also shows that the total time spent in each chamber during the pretest phase did not vary much, resulting in median level pretest CPP scores (Figure 7). These results suggest that the stimuli chosen for the conditioning apparatus were themselves non-biased. The question of whether tactile or visual stimuli should be chosen for conducting a biased CPP paradigm is more so a question of whether a strong initial bias is wanted when conducting a biased CPP paradigm. In the present study, it could be said that a weak initial bias results in less significant findings when comparing pretest and posttest CPP scores. On the other hand, had there been a strong bias for one stimulus over the other, it could be suggested that the stimuli themselves were bias and therefore not a valid measure of drug-induced conditioning. For this study, the stimuli were intended to be distinct from each other but non-biased as to ensure that posttest scores would reflect the change is preference for something the animals were impartial. While this resulted in non-significant changes in pretest vs posttest CPP scores across all conditioning groups, these results may also be due to the dosages of nicotine chosen and/or the time lapse in which animals were injected before being placed into the conditioning apparatus.

A meta-analysis conducted by Matta et al., 2007 on the use of nicotine in *in vivo* research states that peak brain nicotine levels can be observed within 15 minutes of s.c. or i.p. injections. In the present study, animals were injected and then immediately placed in the conditioning apparatus for 20 minutes. It is possible that the full effects of nicotine were only in effect for approximately 10 of the 20 minutes spent in their non-preferred chamber. Due to time constraints during experimentation, immediate placement of animals into the apparatus after injection was a plausible option. Given this, while it is possible that the dosage of 1.2mg/kg (free base) used

could have resulted in CPA, many studies using this dosage reported successful CPP in female and male adult rats (e.g., Matta et al., 2007, Le Foll and Goldberg 2004, Torres et al., 2009). The present study observed increases in CPP scores from pretest to posttest at this dosage, with only 1 animal in the control group exhibiting CPA. It is more likely then that the lack of statistical significance observed when comparing pretest and posttest CPP scores were due to the time lapse in the onset of the drug mentioned prior rather than the dosage used. However, unlike some studies which observed CPA in OVX rats at a dose of 1.2mg/kg (e.g., Torres et al., 2009), OVX rats in the present study did not display CPA and increased in CPP scores from pretest to posttest. However, OVX rats had the smallest increase (Figure 5) and the lowest posttest CPP scores (Figure 4). While it didn't reach statistical significance, OVX animals CPP scores for pretest vs posttest and posttest scores support the hypothesis that ovarian hormones played a role in the controls and ICI's larger CPP scores. The hypothesis that these hormones effects are due to central system activity and not peripheral, are also supported by the results shown in Figure 3, as the difference between ICI's and OVX's reached statistical significance, $p < .05$. However, when observing uterine weight, the hypothesis that both ICI and OVX animals would be lower than controls was not satisfied.

ICI 182,780 was the drug used in the ICI condition group. This drug has been thoroughly researched in humans, rats, and mice and is heavily suggested to be a "pure" peripheral antiestrogen (e.g., Andersson et al., 2005; Howell et al., 2000; Rivera and Eckel, 2010; Song et al., 2019; Wakeling et al., 1991). The purpose of conducting the uterotrophic bioassay was to see if the dosage, route of administration, frequency of administration, and time of metabolism allowed for the drug was successful in inhibiting peripheral estrogen activity during the condition phase. As suggested by Kanno et al., 2001, UT weight is a reliable representation of

relative peripheral estrogen activity, with the lower weight signifying lower activity. As expected, OVX animals displayed lower UT weights compared to controls and ICI's (Figure 8). However, ICI animal's UT weights were much higher than OVX's and close to that of the controls. This could be explained by the fact that the animals were only given the drug once a day, and only during the condition phase. Some animals in this group went without an injection of ICI for a week before perfusion, possibly giving time for peripheral estrogen activity to recover to homeostatic levels. The present study is novel in its use of ICI 182,780 in that most research using the drug did not use it for studying addiction, nor made any mention of the time it takes rats to metabolize the drug after injection, how long it stays active in the body, or its combined effects with nicotine or any other addictive drug. Further research is needed into the specifics of these questions if ICI 182,780 or any other peripheral antiestrogen is to be used in addiction research. Furthermore, the uterotrophic bioassay recorded UT weights as "wet" weights which result in more variability in UT weight (Kanno et al., 2001), as compared to "dry" weights which involve nicking and blotting the uterus after excision. Given these variables, it is impossible to say whether or not observed CPP scores in ICI animals were void of peripheral estrogen activity. However, the methods in which the drug was used in this study and the questions posed here give insight to possible future research using this drug.

Despite the uncertainty of the specifics of how ICI 182,780 should have been used in an addiction study, the results produced by the ICI animals fit the present hypotheses of expected CPP scores. Figures 4 and 5 show that CPP scores for the ICI animals were very similar to the controls and different from OVX animals. These results suggest that if ICI 182,780 was functioning as intended (e.g., blocking peripheral, but not central estrogen activity) during conditioning, it is possible that the results of this study make plausible the theory that heightened

addictive potential in female rats is due solely to central nervous system activity of estrogen. Further results of the present study help support this theory. The results of the OVX animals CPP scores when compared to controls and ICI's were lower in the posttest and had less of a change when comparing pretest vs posttest CPP scores. Evidence that ovariectomy surgeries were successful in complete removal of ovaries and thus complete removal of estrogen activity in the whole body are shown in the CPP scores of the OVX animals, as well as the drastic increase in body weight for these animals post-surgery (Figure 3). It is known that estrogen is involved in the regulation of metabolism and body weight in the central nervous system. Studies suggest that these functions effect eating behavior in both sexes and stimulate brown adipose tissue thermogenesis (Xu and Lopez, 2018). Because OVX animals were the only group to display a large increase in body weight and had the lowest UT weights (a correlate of peripheral estrogen activity as mentioned prior), is it likely that ovariectomy surgeries were successful in completely eliminating estrogen production and activity in the body.

Locomotion activity was not intended to be analyzed in the present study, but because of the large increase in entrances across all conditioning groups, it was worth noting. Comparing the total number of entrances animals made into the conditioning chambers (not including the middle chamber) made strong evidence for an increase in locomotor activity following the condition phase. Even with only 3 total s.c. injections of nicotine per animal during conditioning, most animals doubled or tripled their number of entrances. While video tracking was not used during the pretest or posttest, however it was observed during these tests that animals in all conditioning groups went from calmly exploring the conditioning apparatus during the pretest to sprinting between chambers during the posttest. Studies have found that nicotine increases

locomotor activity in male rats across various age groups and doses (Beluzzi et al., 2003), with some reporting greater results with s.c. injections rather than i.p. (Ahsan et al., 2014).

While many of the results presented support the hypotheses of this study, there was a lack of statistical significance for most of them. However, this lack of significance could be explained mostly by small sample sizes. While small sample sizes were required to be able to complete the study due to time and resource constraints, they resulted in less statistical power for each conditioning group. Furthermore, because times and entrances during the pretest and posttest were recorded by hand, human error may have accounted for slightly inaccurate counts of both of these measures, resulting in inaccurate CPP scores. Most studies using a CPP paradigm usually use video recording for pretest and posttests, allowing for more precision in time and placement measurements. If this study were to be replicated, it is suggested that a video recording device be used for data collection during pretest and posttest. It is also suggested that sample sizes be increased to n=10 or higher per conditioning group.

The results of this study give possibilities for future research. Research should be done on the effects of having a biased or non-biased stimuli in the conditioning apparatus as to call in to question the legitimacy of prior research done using such stimuli. Future research should also be done on how ICI 182,780 can be used effectively in an addiction study to be able to more accurately discriminate between peripheral and central nervous system estrogen activity. If these differences can be more easily assessed, perhaps research into central antiestrogens could be useful into aiding in treating addiction in females.

References

Abuse, National Institute on Drug.Tobacco/nicotine and E-cigs. Retrieved from https://www.drugabuse.gov/drugs-abuse/tobacconicotine-e-cigs

Ahsan, H. M., de la Peña, June Bryan I, Botanas, C. J., Kim, H. J., Yu, G. Y., & Cheong, J. H. (2014). Conditioned place preference and self-administration induced by nicotine in adolescent and adult rats. *Biomolecules & Therapeutics, 22*(5), 460-466. doi:10.4062/biomolther.2014.056

Allen, A., Oncken, C., & Hatsukami, D. (2014a). Women and smoking: The effect of gender on the epidemiology, health effects, and cessation of smoking. *Current Addiction Reports, 1*(1), 53-60. doi:10.1007/s40429-013-0003-6

Allen, A. M., Lunos, S., Heishman, S. J., al'Absi, M., Hatsukami, D., & Allen, S. S. (2014b). Subjective response to nicotine by menstrual phase. *Addictive Behaviors, 43*, 50-53. doi:10.1016/j.addbeh.2014.12.008

Andersson, N., Ulrika Islander, Emil Egecioglu, Elin Löf, Charlotte Swanson, Sofia Movérare-Skrtic, . . . Claes Ohlsson. (2005). Investigation of central versus peripheral effects of estradiol in ovariectomized mice. *Journal of Endocrinology, 187*(2), 303-309. doi:10.1677/joe.1.06181

Becker J.B. (1999). Gender differences in dopaminergic function in striatum and nucleus accumbens.. *Pharmacology Biochemistry and Behavior, 64*(4), 803-812. doi:10.1016/S0091-3057(99)00168-9

Becker, J. B., & Rudick, C. N. (1999). Rapid effects of estrogen or progesterone on the amphetamine-induced increase in striatal dopamine are enhanced by estrogen priming: A microdialysis study. *Pharmacology, Biochemistry and Behavior, 64*(1), 53-57. doi:10.1016/S0091-3057(99)00091-X

Belluzzi, J., Lee, A., Oliff, H., & Leslie, F. (2004). Age-dependent effects of nicotine on locomotor activity and conditioned place preference in rats. *Psychopharmacology, 174*(3), 389-395. doi:10.1007/s00213-003-1758-6

Benowitz, N. L. (2010). Nicotine addiction. *The New England Journal of Medicine, 362*(24), 2295-2303. doi:10.1056/NEJMra0809890

Brielmaier, J. M., McDonald, C. G., & Smith, R. F. (2008). Nicotine place preference in a biased conditioned place preference design. *Pharmacology, Biochemistry and Behavior, 89*(1), 94-100. doi:10.1016/j.pbb.2007.11.005

Cross, S. J., Linker, K. E., & Leslie, F. M. (2017). Sex-dependent effects of nicotine on the developing brain. *Journal of Neuroscience Research, 95*(1-2), 422-436. doi:10.1002/jnr.23878

CDCa Current cigarette smoking among adults in the united states. (2019). Retrieved from https://www.cdc.gov/tobacco/data_statistics/fact_sheets/adult_data/cig_smoking/index.htm

CDCb Fast facts. (2019). Retrieved from https://www.cdc.gov/tobacco/data_statistics/fact_sheets/fast_facts/index.htm

CDCcYouth and tobacco use. (2019). Retrieved from https://www.cdc.gov/tobacco/data_statistics/fact_sheets/youth_data/tobacco_use/index.htm

Curtis, L., Buisson, B., Bertrand, S., & Bertrand, D. (2002). Potentiation of human α4β2 neuronal nicotinic acetylcholine receptor by estradiol. *Molecular Pharmacology, 61*(1), 127-135. doi:10.1124/mol.61.1.127

Harrod, S. B., Booze, R. M., & Mactutus, C. F. (2006). *Sex differences in nicotine levels following repeated intravenous injection are attenuated by gonadectomy in female rats* doi:10.1016/j.pbb.2006.12.004

Health, National Center for Chronic Disease Prevention and Health Promotion (US) Office on Smoking and. (2014). *Patterns of tobacco use among U.S. youth, young adults, and adults* Centers for Disease Control and Prevention (US). Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK294302/

Howell, A., Osborne, C. K., Morris, C., & Wakeling, A. E. (2000). ICI 182,780 (faslodex): Development of a novel, "pure" antiestrogen. *Cancer, 89*(4), 817. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10951345

Hu, M., Watson, C. J., Kennedy, R. T., & Becker, J. B. (2006). Estradiol attenuates the k+-induced increase in extracellular GABA in rat striatum. *Synapse, 59*(2), 122-124. doi:10.1002/syn.20221

Is vaping safer than smoking cigarettes? (2016, -05-22T15:23:51+00:00). Retrieved from http://www.center4research.org/vaping-safer-smoking-cigarettes-2/

Jones, L. (2018, -05-31). Vaping - the rise in five charts. Retrieved from https://www.bbc.com/news/business-44295336

Kanno, J., Lesley Onyon, Joseph Haseman, Penelope Fenner-Crisp, John Ashby, & William Owens. (2001). The OECD program to validate the rat uterotrophic bioassay to screen compounds for in vivo estrogenic responses: Phase 1. *Environmental Health Perspectives, 109*(8), 785-794. doi:10.1289/ehp.01109785

Le Foll, B., & Goldberg, S. (2005). Nicotine induces conditioned place preferences over a large range of doses in rats. *Psychopharmacology, 178*(4), 481-492. doi:10.1007/s00213-004-2021-5

Loria, K.Use of E-cigarettes among teens is 'exploding'. Retrieved from https://www.consumerreports.org/electronic-cigarettes/e-cigarette-use-among-teens-is-exploding/

Lynch, W. J., & Sofuoglu, M. (2010). Role of progesterone in nicotine addiction. *Experimental and Clinical Psychopharmacology, 18*(6), 451-461. doi:10.1037/a0021265

Matta, S. G., Balfour, D. J., Benowitz, N. L., Boyd, R. T., Buccafusco, J. J., Caggiula, A. R., . . . Zirger, J. M. (2007). Guidelines on nicotine dose selection for in vivo research. *Psychopharmacology, 190*(3), 269-319. doi:10.1007/s00213-006-0441-0

Natarajan, R., Wright, J. W., & Harding, J. W. (2011). Nicotine-induced conditioned place preference in adolescent rats. *Pharmacology, Biochemistry and Behavior, 99*(3), 519-523. doi:10.1016/j.pbb.2011.05.004

National Institute on Drug Abuse, (NIDA), (2020).Vaping devices (electronic cigarettes). Retrieved from https://www.drugabuse.gov/publications/drugfacts/vaping-devices-electronic-cigarettes

Printz, C. (2015). Lung cancer new leading cause of death for women in developed countries: Data reflects increased rates of smoking. *Cancer, 121*(12), 1911-1912. doi:10.1002/cncr.28995

Rivera, H. M., & Eckel, L. A. (2010). Activation of central, but not peripheral, estrogen receptors is necessary for estradiol's anorexigenic effect in ovariectomized rats. *Endocrinology, 151*(12), 5680-5688. doi:10.1210/en.2010-0731

Smith, P. H., Kasza, K. A., Hyland, A., Fong, G. T., Borland, R., Brady, K., . . . McKee, S. A. (2015). Gender differences in medication use and cigarette smoking cessation: Results from the international tobacco control four country survey. *Nicotine & Tobacco Research : Official Journal of the Society for Research on Nicotine and Tobacco, 17*(4), 463-472. doi:10.1093/ntr/ntu212

Song, Z., Yang, H., Peckham, E. M., & Becker, J. B. (2019). Estradiol-induced potentiation of dopamine release in dorsal striatum following amphetamine administration requires estradiol receptors and mGlu5. *eNeuro, 6*(1), ENEURO.0446-18.2019. doi:10.1523/ENEURO.0446-18.2019

Sun, Y., Chen, G., Zhou, K., & Zhu, Y. (2018). A conditioned place preference protocol for measuring incubation of craving in rats. *Journal of Visualized Experiments,* (141) doi:10.3791/58384

Torres, O. V., Natividad, L. A., Tejeda, H. A., Van Weelden, S. A., & O'Dell, L. E. (2009). Female rats display dose-dependent differences to the rewarding and aversive effects of nicotine in an age-, hormone-, and sex-dependent manner. *Psychopharmacology, 206*(2), 303-312. doi:10.1007/s00213-009-1607-3

Wakeling, A. E., Dukes, M., & Bowler, J. (1991). A potent specific pure antiestrogen with clinical potential. *Cancer Research, 51*(15), 3867. Retrieved from http://cancerres.aacrjournals.org/cgi/content/abstract/51/15/3867

Weinberger, A. H., Smith, P. H., Allen, S. S., Cosgrove, K. P., Saladin, M. E., Gray, K. M., . . . McKee, S. A. (2015). Systematic and meta-analytic review of research examining the impact of menstrual cycle phase and ovarian hormones on smoking and cessation. *Nicotine & Tobacco Research : Official Journal of the Society for Research on Nicotine and Tobacco, 17*(4), 407-421. doi:10.1093/ntr/ntu249

Wetherill, R., Franklin, T., & Allen, S. (2016). Ovarian hormones, menstrual cycle phase, and smoking: A review with recommendations for future studies. *Current Addiction Reports, 3*(1), 1-8. doi:10.1007/s40429-016-0093-z

Xu, Y., & López, M. (2018). Central regulation of energy metabolism by estrogens. *Molecular metabolism*, *15*, 104–115. https://doi.org/10.1016/j.molmet.2018.05.012

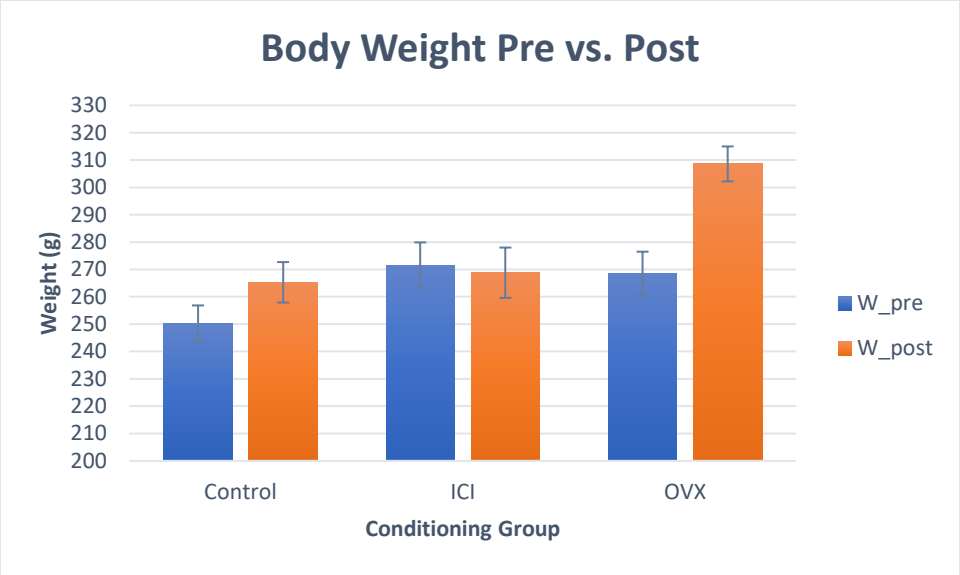


Fig.3. Comparison of each conditioning groups body weight (g) from the pretest to the posttest of the conditioning phase. The change in body weight was significant between controls and OVX rats, $p<.05$. Error bars represent Std. Error.

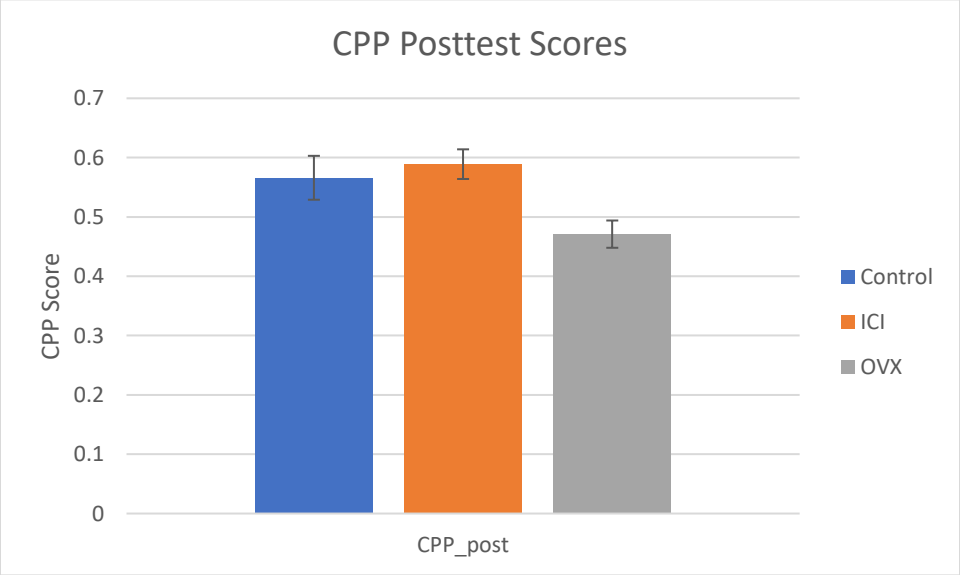


Fig.4. Comparison of CPP scores from the posttest. The difference between ICI's and OVX's were significant, $p<.05$. Error bars represent Std. Error.

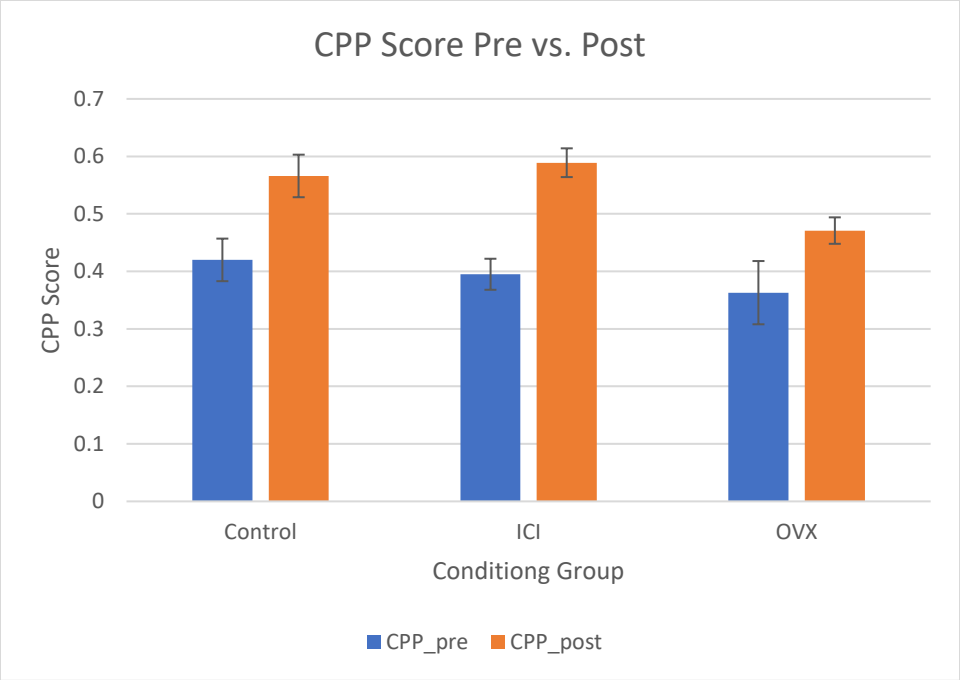


Fig.5. Comparison of each condition groups pretest and posttest CPP scores. No differences reached a significance of $p<.05$. Error bars represent Std. Error.

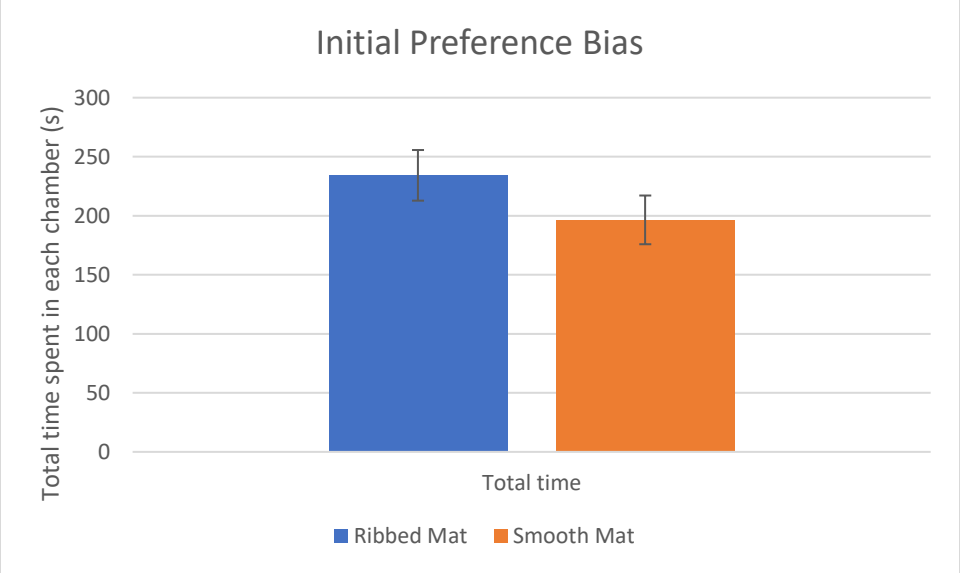


Fig.6. Comparison of total time (s) spent in conditioning chamber during the pretest of the conditioning phase. Times are the combination of the times from all 3 conditioning groups. Error bars represent Std. Error.

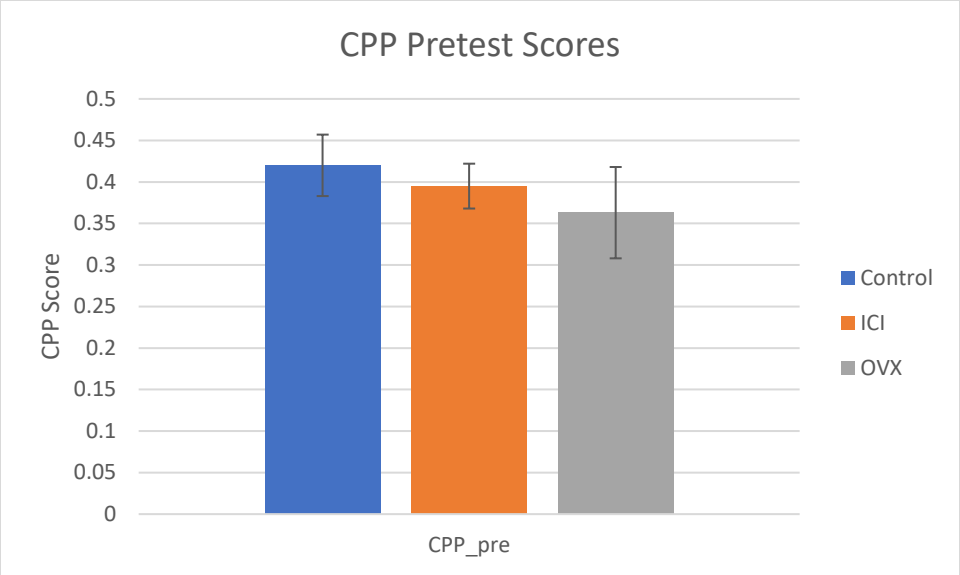


Fig.7. Comparison of pretest CPP scores. Scores represent the relative strength of each conditioning group to their initially preferred chamber. Error bars represent Std. Error.

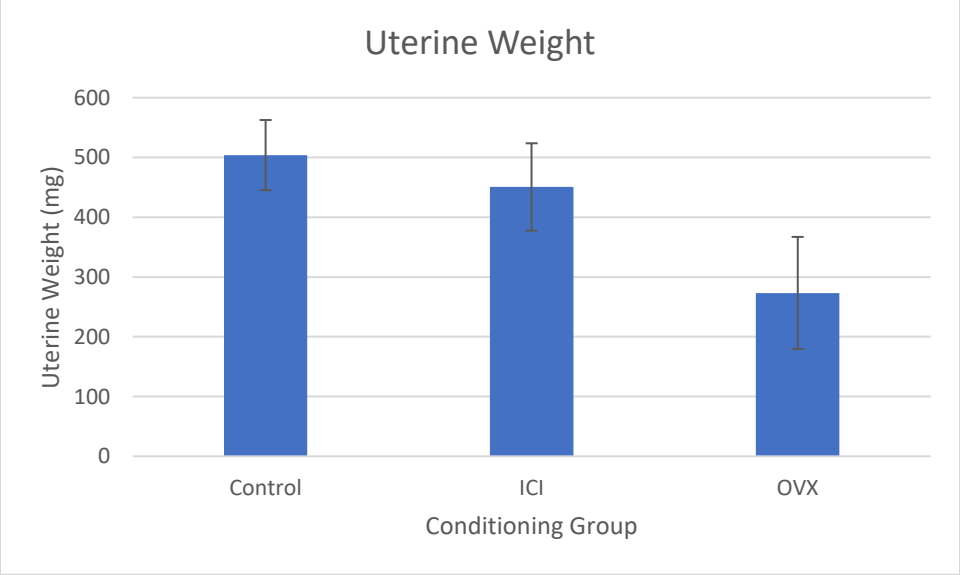


Fig.8. Comparison of uterine weight (mg) (after perfusion) across conditioning groups. Lower uterine weight signifies less peripheral estrogen activity. Error bars represent Std. Error.

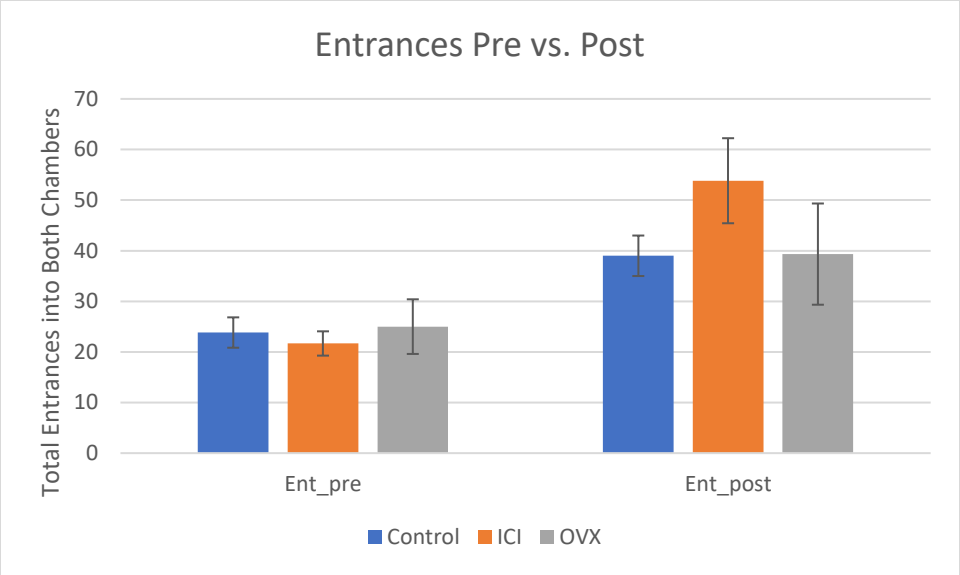


Fig.9. Comparison of total # of entrances into either condition chamber during the pretest and posttest. This is showing the change of each conditioning groups relative locomotor activity over the course of the conditioning phase. The difference in entrances from significant from the pretest to posttest, $p<.001$.