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Examining the Rapid Non-Classical Effects of 17β -Estradiol on Sexual Advertisement Behavior of the Golden Hamster (*mesocricetus auratus*)

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Female Syrian hamsters engage in vaginal scent-marking (VM) to advertise their sexual receptivity. VM is facilitated by estrogen; however, the hormone's mechanism of action is unknown. Classically, estrogen binds to intracellular receptors and promotes gene transcription to eventually affect behavior. Estrogen may non-classically and rapidly affect behavior via binding to membrane localized receptors. In order to determine how estrogen modulates VM, VM levels were examined in 10 female hamsters across several hormone conditions. Levels of VM were assessed across 3 trials in intact, normally-cycling subjects. These trials were repeated following ovariectomy. A final set of trials were conducted in ovariectomized subjects injected with either 1β -estradiol or vehicle and tested 30 minutes following injection. VM frequency significantly decreased after ovariectomy. There was no significant effect, however, of 1β -estradiol compared to vehicle injections on VM behavior. These results suggest that estradiol may not regulate vaginal marking via a non-classical mechanism. KEYWORDS: Vaginal marking, Syrian Hamster, GPR30, GPER, Sexual behavior, 1β-estradiol

Attracting mates and promoting sexual receptivity are effective methods for increasing an organism's reproductive fitness; moreover, performing precopulatory appetitive behaviors will inevitably benefit the species (Robert E. Johnston, 1977). Female Syrian hamsters perform a specific appetitive behavior that utilizes chemosensory cues to inform male counterparts of their impending receptivity. This behavior, known as vaginal marking, is a scent marking behavior which is characterized by the hamster's application of vaginal secretion onto a substrate by lowering their anogenital region onto said substrate, deflecting their tail upward, and expressing forward movement. There are several known external factors that cause female hamsters to vaginal mark (e.g. male odors, proximity of alpha males, absence of predators) (Huck, Lisk, & Gore, 1985a; Robert E. Johnston, 1977; Steel, 1985); however, the internal processes whereby neuronal factors facilitate vaginal marking are still unclear.

Ethologically, the vaginal secretion that is deposited by the female during a vaginal mark is attractive to opposite-sex conspecifics (Johnston & Lee, 1976; Takahashi & Lisk, 1983). In fact, vaginal secretion is the most attractive odor to sexually naïve male hamsters when compared to other odors available from female subjects (e.g. flank, saliva) (Johnston, 1974). Males have also been found to vocalize more frequently when they were investigating an area that had recently been

vaginally scent marked by a female (Johnston & Kwan, 1984). Kwan (1980) discusses that vaginectomized females—removed of their ability to deposit vaginal secretions—are less appealing to males than intact females even when contact was prevented. Justifiably, males prefer odors of females that can deposit vaginal secretions when compared to odors from females that cannot. Johnston (1976) found that conspecific preference of scent marking odors remained intact for up to 25 days; moreover, he theorized that the function of flank and vaginal marking behaviors were prosocial. Steel (1985) asserts that the functions of vaginal marking behaviors are sexually specific; consequently, she found that vaginal marking is significantly correlated with impending sexual receptivity and male proximity. In fact, female hamsters preferentially vaginal mark in the area between possible mates and the female's home burrow; suggesting that females vaginal mark in order to lead males toward the female's nest (R. D. Lisk, Ciaccio, & Catanzaro, 1983). This body of evidence suggests that female hamsters generally vaginal mark proximally to males in order to attract potential mates; therefore, female hamsters perform their highest levels of vaginal marking while being presented with conspecific odors.

Petrulis and Johnston (1997) suggest that females perform significantly more vaginal marks when they are presented with odors specifically from the flank and rump of conspecifics compared to females not presented with these odors. Female hamsters also vaginal mark more frequently than solitary controls in the proximity of conspecifics and preferentially in the territory of alpha males (Huck, et al., 1985a; Steel, 1985). Steel (1985) specifically found that females vaginal mark more often while being actively followed by a male. These findings suggest that females vaginally scent mark more frequently when they detect the presence of possible mates.

Female hamsters most frequently vaginal mark on the third day of their four day estrous cycle, proestrus, 24 hours prior to the onset of sexual receptivity (Johnston, 1979; Steel, 1985). Johnston (1977) found that proestrous females vaginal mark significantly more when exposed to a male odor than on any other day of the animals' cycle. Again, the timing of this increase in vaginal marking frequency is suggested to illustrate the animal's advertisement of impending sexual receptivity. Additionally, proestrus is the day of the animal's 4-day cycle in which estrogen levels are at their highest (Robert D. Lisk & Nachtigall, 1988). In light of this evidence, vaginal marking is regulated both by male odors and endogenous estrogen levels in the physiological system of the female hamster; in fact, estrogen levels have been found to be positively correlated with vaginal marking frequency across the animal's four day estrous cycle (Robert D. Lisk & Nachtigall, 1988; Steel, 1985). To specifically examine the effects of estrogen on sexual behavior, Burnett, et al. (1985) manipulated estrogen levels in brain regions associated with female golden hamster sexual behavior and found that estrogen facilitates precopulatory behavior and is implicated in the regulation of prosocial behavior. Takahashi (1990) further asserts that abolishing vaginal marking behavior—by complete bilateral ovariectomy—and subsequent exogenous estrogen treatments completely restore vaginal marking in response to conspecific odors. It is clear from these observations that sexually appetitive vaginal marking behavior of the Syrian hamster is strongly modulated by estrogen (Been & Petrulis, 2008; Robert D. Lisk & Nachtigall, 1988). Taken together, these data suggest that estradiol acts within target structures to regulate the expression of vaginal marking in female hamsters; however, the hormone's mechanism of action is still unknown.

Classically, estrogen is believed to exhibit its effects on sexual behavior by binding to intracellular nuclear receptors and subsequently activating gene transcription (L. K. Takahashi, 1990; Vasudevan, Kow, & Pfaff, 2001). The classical effects of estrogen occur over periods of hours, not minutes; in other words, estrogen must rely on protein synthesis and RNA transcription to affect behavior because, at the time of classical behavioral effects, exogenous estrogen treatment in ovariectomized animals is believed to have already cleared from the physiological system (McEwen, 1988). The classical effects of estrogen on the brain occur slowly via synaptogenesis, altering neuronal plasticity, and neuronal growth.

More recently, evidence has been

presented for the non-classical action of such hormones (Toran-Allerand, et al., 1999; Vasudevan & Pfaff, 2007). Cross & Roselli (1999) found that 17²˜ ” ˇ!ÿ¨ˇ-estradiol, the most potent type of endogenous estrogen, affects sexual behavior in male rats within 15 minutes of its administration. This is believed to be an insufficient amount of time for gene transcription to take place; therefore, this rapid effect of 17β-estradiol on male sexual behavior is believed to be a result of a non-classical action of steroid hormones within the physiological system. In his review of the non-classical effects of estrogens, King (2008) describes several novel mechanisms by which steroid hormones facilitate sexual behavior. King (2008) further suggests that membrane-localized sex hormone receptors in the brain mediate the rapid hormone actions that affect female reproductive behavior. Canonaco et al. (2008) provide specific evidence for the co-expression of estrogen in a G-protein coupled receptor (GPR30) in female golden hamster brain regions associated with sexual behavior.

In fact, the expression of estrogen via GPR30 is well-established and distinct from classical effects of estrogen via intercellular estrogen receptors; consequently, GPER (g-protein estrogen receptor) is now the common acronym for this membrane receptor because of its well-established connection with estrogen (Maggiolini & Picard, 2009). Furthermore, Gu and Moss (1998) elaborate on the theory that non-classical action of estrogen occurs through the potentiation of kinate-induced currents in g-protein coupled receptors. In fact, there are multiple novel routes by which estrogen may rapidly activate neural activity (Toran-Allerand, 2004).

Vasudevan and Pfaff (2001) investigated the relationship between classical and non-classical effects of estrogen and discovered that classical gene expressional effects occurred as a result of rapid non-classical potentiation of the cellular membrane. 17β -estradiol has been shown to rapidly and non-classically alter membrane conductance and thus elicit gene transcription; this process is suggested to regulate sexual behavior of female rats and possibly other rodents (Vasudevan, Kow, & Pfaff, 2005). Vasudevan and Pfaff (2007) surmise that rapid membrane effects of estrogen facilitate classical gene transcription by activating a cascade of protein kinases which release calcium and activate said transcription; however, rapid non-classical effects of estrogen have been strongly implicated in regulating sexual behavior independently from gene transcription (Cross & Roselli, 1999; Micevych, et al., 2007).

The medial amygdala is implicated in integrating behavioral responses with social odors in the golden hamster; therefore, it is no surprise that lesions to this area substantially reduce vaginal marking in response to conspecific odors (Petrulis & Johnston, 1999). 17β-estradiol has been found to exhibit rapid effects on medial amygdaloid neurons even after suppression of protein synthesis (Nabekura, Oomura, Minami, Mizuno, & Fukuda, 1986). Hyperpolarization of medial amygdala neurons occurred in brain slices after being incubated in a protein synthesis inhibition solution and when immediately exposed to 17β-estradiol. Increased membrane conductance and hyperpolarization of post-synaptic cells were observed significantly more in females brain slices than in males. Nabekura et al. (1986) concluded that 17β -estradiol activated medial amygdaloid neurons specifically by means of non-classical rapid hormone action. This is interesting because 17β-estradiol is more endogenously abundant, and more closely involved with sexual behavior in females than in males. Also, 17β-estradiol has been found to rapidly activate brain regions associated with vaginal marking behavior in response to male odors. Other researchers have more recently found that rapid effects of estradiol on cellular mechanisms are independent of classical pathways (Gu, Korach, & Moss, 1999). Researchers have further asserted that estrogen, recently found in high concentrations in extracellular regions where it classically would not exhibit neuronal control, rapidly affects neuronal excitation, plasticity, and synaptic transmission similarly to neurotransmitters (Woolley, 2007). This evidence suggests that estrogen, particularly 17 β-estradiol, rapidly acts on the brain in many novel ways. Vaginal marking of the golden hamster is a stereotyped behavior that is closely modulated by estrogen; thus, researchers have observed changes in this behavior after artificially controlling

estrogen in an animal model (Robert D. Lisk & Nachtigall, 1988). Classically, estrogen capsules or lipid soluble estrogen benzoate injections, following ovariectomy, were administered subcutaneously to elicit estrogen to classically modulate sexual behavior (Robert D. Lisk & Nachtigall, 1988; L. K. Takahashi, 1990); however, rapid effects of estrogen on sexual behavior could not be examined by means of subcutaneous implant or estrogen benzoate injections. Rapid introduction of 17β-estradiol via injection, and subsequent observation of vaginal marking behavior within a matter of 15 minutes, would strictly reflect non-classical effects of estrogen on the animal's behavior. Estrogen and other steroid hormones, however, are lipid soluble; therefore, an injection vehicle must be used to rapidly dissolve estrogen in an hamster's intraperitoneal cavity—a water soluble environment—in order to examine the non-classical effects of the introduced estrogen on sexual behavior (Mark G. Packard & Teather, 1997).

Previously, Packard & Teather (1997) dissolved estrogen in a 2-hydroxyproyl- β-cyclodextrin (CDX) solubility solution in sterile saline and injected their solution into a rodent's intraperitoneal cavity, and observed rapid behavioral effects of estrogen in their rodent model. Furthermore, testosterone has been dissolved in CDX and administered by subcutaneous injections, and was found to mimic endogenous release of hormones by the testes in male rats. Also, CDX alone is non-toxic, and does not affect behavior, when injected over a 16-week period (Cross & Roselli, 1999; Pitha, 1985). In summation, CDX has been shown to increase the solubility of steroid hormones and does not produce behavioral effects individually, thereby providing a stable and supportive soluble vehicle for steroid hormones to exhibit their well documented rapid affects on the physiological system of an awake behaving animal (M. G. Packard, Cornell, & Alexander, 1997).
Cross and Roselli observed effects of 100 μg/kg of 17β-estradiol in a CDX on sexual behavior of male rats within 15 minutes of administration. By following this successful comparative procedure, the present study investigates the rapid effects of 17 β-estradiol on vaginal marking behavior of the golden hamster. We will administer this dosage of 17β-estradiol to ovariectomized female hamsters exposed to male odors and examine their subsequent vaginal marking behavior in order to observe any strictly rapid effects of estrogen on vaginal marking. Because of the recent findings supporting novel action of estrogens and the well documented correlation between estrogen and appetitive behavior, we hypothesize that 17β-estradiol—administered via intraperitoneal injection in CDX vehicle—will restore vaginal marking behavior 30 minutes following injection in ovariectomized subjects, through a rapid, non-classical mechanism.

Method

Subjects

Female golden hamsters (*Mesocricetus auratus*), 70-120 days of age, were observed within eight total vaginal marking trials over several weeks (n=10, 106-168g). A separate group of 28 male golden hamsters, over 60 days of age, provided odor donation for female subjects in order to elicit female vaginal marking during behavioral trials. Females were unrelated to, and had not previously encountered, the odor from any male odor donor prior to this study; furthermore, females were never presented with the same male odor more than once across all trials. Female subjects as well as male odor donation animals were sexually naïve prior to and throughout the present study.

All female subjects were bred in-house from golden hamsters purchased from Charles River Laboratory; female subjects were group-housed from birth with four to five female cage mates until they reached 65 days of age. After 65 days, subjects were individually housed in solid bottom Plexiglas cages (42 x 21 x 21cm) throughout the course of the experiment; food and water were available *ad libitum*. Odor donor stimulus male animals were purchased directly from Charles River Laboratory and cared for under comparable conditions. All animals were maintained on a reversed 14:10 light/dark photoperiod. Female subjects were housed within a mixed-sex species specific room with up to 80 other Syrian hamsters (seven of which were male

odor donors). Other male donors were held in separate rooms and maintained under identical conditions and on a similar light/dark photoperiod. All behavioral trials were conducted at the beginning of the female subjects' dark photoperiod in order to observe the highest frequencies of vaginal marking per animal (Huck, et al., 1985a).

Vaginal marking behavioral trials were conducted on the third day (proestrus) of their four day estrous cycle, 24 hours prior to sexual receptivity. Estrous cycles were determined by observing the consistency of the subjects' vaginal secretions over the course of 8-10 consecutive days. For this procedure, subjects were held securely and gentle pressure was applied to their anogenital region with a disposable probe in order to promote the expulsion of vaginal secretion. Vaginal secretion varies in consistency across the animal's estrous cycle; stringy secretions indicate that the animal is in behavioral estrus (Orsini, 1986). Behavioral estrus was further verified by observing lordosis (sexual receptivity posture specific to estrous) after the female was temporarily placed in a stimulus male's home cage. The stimulus males quickly and efficiently elicited lordosis from the receptive female subjects on the predicted day of behavioral estrous; subsequently, the female was quickly removed from the cage to prevent sexual experience. These exposures occurred only when we observed stringy vaginal secretions, and in equal frequencies across subjects; furthermore, this temporary exposure was the only physical experience female subjects had with conspecifics. All procedures were approved by the Institutional Animal Care and Use Committee at Georgia State University.

Testing Apparatus

Vaginal marking trials were conducted in an odor donation male's vacated cage. Male odor donation animals inhabited a typical cage for a 7 day period leading up to the time of the experimental trial. During this period, males dispersed their odor throughout the cage by means of scent marking and normal habitation over time. All cages contained corncob litter and 12g of cotton bedding (4 Nestlets, ANCARE, Bellmore, NY). Odor donation cages were carefully removed of aversive or confounding odor sources (e.g. urine, food) and the soiled cotton bedding was carefully separated into a rectangular shape and laid flat underneath a perforated and painted Plexiglas vaginal marking plate (40cm x 19cm x 6mm, see Figure 1).

The vaginal marking plate, consuming roughly 85% of the floor surface space of the cage, was centered between the walls and laid flat on top of the cotton bedding and corncob litter. There were 105 perforations spaced evenly throughout the plate, each with an individual diameter (~2mm): large enough to passively diffuse odor contained in the soiled bedding underneath. Flat black spray paint was applied to the majority of the marking plate with unpainted lines that were used to quantify locomotion during behavioral trials (see Figure 1). The vaginal marking plate served several functions in this experiment; firstly, the plate provided a visibly contrasting and minimally intrusive substrate for experimenters to reliably and accurately observe vaginal marking and locomotion behavior; in addition, it limited the female subject's physical exposure and disruption of the stimulus male's soiled cotton bedding underneath. Several identical plates were used throughout the experiment; plates used multiple times throughout the day were cleaned with 70% alcohol solution between trials.

Surgery and Estradiol Injections

In order to control circulating levels of estradiol in the animal subjects, ovariectomy (OVX) surgery was performed on all animals after baseline levels of vaginal marking were observed across three Pre-OVX trials. All female subjects were maintained under 1-2% isoflourine anesthesia (100% oxygen) during OVX surgery. Their ovaries were removed through two bilateral incisions on the medial-dorsal surface of the anesthetized animals; wound clips were used to close the incisions. Ketoprofen (5mg/kg) was administered as a post-op analgesic via subcutaneous injection. One ml of sterile saline was also administered post-op via subcutaneous injection in order to supplement the animals' lost fluids during surgery. Animals began Post-OVX behavioral trials after seven days of a surgical recovery period.

Estradiol, via intraperitoneal injections, was administered to ovariectomized animals and their subsequent behavior was observed in order to

examine the rapid effects of estradiol on vaginal marking behavior. Experimental injections consisted of β-Estradiol minimum 98% (Sigma Aldrich) dissolved in a 20% aqueous C2-Hydroxypropyl- μCyclodextrin (Sigma Aldrich) sterile saline solution, at a dosage of 100 μg/kg. The 20% Cyclodextrin saline vehicle served as the solution for our control injections. Experimental and control injections were counterbalanced across two drug trials during these procedures. The researcher scoring vaginal marking behavior in both drug trials was aware of the identity of the injections, but blind to which injection the animals received. The researcher administering the estradiol and vehicle injections was blind to the identity of the injections, and did not participate in scoring behavior for these trials.

Behavioral Testing

Behavior was observed across three identical 10-minute trials on proestrous days, an additional three trials following ovariectomy surgery, and finally, two trials 30 minutes after estradiol or vehicle injections. During all trials, female hamsters were placed in the testing apparatus (see above) in a red-lighted room to simulate the animal's dark photoperiod; the red light is not visually perceivable to hamsters but is bright enough for experiments to observe animal behavior during trials. Experimenters observed the animals for vaginal marking frequency, latency to vaginal mark, and frequency of line crosses. Vaginal marking frequency was counted by experimenters each time the animal pressed their rump onto the marking plate, deflected their tail upward, and expressed forward movement. Latency to vaginal mark was recorded as the elapsed time between the beginning of the trial and the when the animal's first vaginal mark was scored. A line cross, representing quantitative locomotion, was scored when an animal passed more than 50% of their body over the central line on the marking plate. Multiple animals that were tested on the same day were consecutively tested in random order.

Data Analysis

Vaginal marking behavior was quantified both by frequency and by latency. Vaginal marking frequency, latency, and line crosses served as this present study's dependent variables. Vaginal marking frequency is a highly variable behavior measure between animals; therefore, a within-subjects design was used in order to reduce confounding variability. In order to determine if differences existed across subjects and trials, multiple one-way design omnibus ANOVAs, with individual animal's vaginal marking frequency scores or line cross scores as different within-subjects factors and trial condition (Pre-OVX, Post-OVX, Drug, Vehicle) as the between-subjects factors, were performed. If significant F values existed, pairwise within-subjects comparisons were made across trial conditions. Other independent variables (e.g. scoring experimenter, testing room) were also examined as possible modifiers of dependent outcomes with multiple independent samples t-tests. Pearson's r was used to identify the relationship between vaginal marking frequency and line cross scores.

Results

The omnibus ANOVA comparing vaginal marking behavior within-subjects revealed a significant difference across trial conditions, $F(3,24) = 78.74$, $p < .01$. Subsequent pairwise comparisons of vaginal marking frequency yielded several significant differences across conditions after Bonferonni correction (see Figure 2).

Pre-OVX vaginal marking frequency was statistically different from all other trial conditions (Post-OVX, Estradiol, Vehicle), $p < .05$; however, there was no difference between vaginal marking frequency scores in estradiol injected animals when compared to animals that received vehicle injections, $p > .05$. In addition, post-OVX vaginal marking frequency scores were not statistically different from frequency scores in either the estradiol or vehicle conditions, $p > .05$. Although a slight reduction line crosses was observed in post-OVX trials, a second omnibus ANOVA, comparing line cross scores within-subjects across all trials, did not reveal any significant difference between trial conditions $F(3,24) = 40.962$, $p = .06$. No subsequent pairwise comparisons were made regarding line cross scores across conditions. Table 1 summarizes all behavior measures across trial conditions.

Other independent variables were considered as possible modifiers of vaginal marking behavior. Although they were

balanced across conditions, there was a significant difference between the two experimenters' reports of vaginal marking frequency, $p < .05$. No difference existed between behaviors scored in different testing rooms, $p > .05$; however, this variable was balanced across conditions. Also, line crosses were positively correlated with vaginal marking frequency in within pre-OVX trials, $r = .60$, $p < .01$, $n = 27$ (see Figure 3a) ; however, this correlation did not exist in any trial after ovariectomy, $r = .09$, $p > .05$, $n = 45$ (see Figure 3b).

Discussion

Contrary to our hypothesis, vaginal marking frequency was not significantly different between the experimental and the control condition. In other words, intraperitoneal injection of 17β-estradiol does not affect golden hamster vaginal marking behavior within 30 minutes of administration; more generally, this suggests that 17β-estradiol does not strictly facilitate vaginal marking behavior via a non-classical mechanism. In support of these findings, there is evidence that suggests classical mechanisms of estrogen are necessary to activate non-classical mechanisms in order for estrogen to rapidly affect behavior (Vasudevan, et al., 2001). Therefore, it is possible that non-classical rapid mechanisms of estradiol are dependent on low levels of circulating estrogen. Thus, estrogen may modulate golden hamster vaginal marking behavior classically or in combination with novel non-classical rapid mechanisms. In this respect, future research should examine the specific relationship between classical and non-classical mechanisms of hormone action.

The present results are also consistent with the conclusion that estrogen does not modulate vaginal marking behavior in a rapid, non-classical manner. If this is indeed the case, it suggests that sexual motivation is not dynamically or non-classically controlled by such systems in Syrian hamsters. Rather, dynamic modulation of appetitive sexual behaviors may instead occur in response to social odors. Social odor signals vary widely across the natural environment of the Syrian hamster; therefore, it may be advantageous for females to regulate their scent marking rapidly depending on the specific signals they encounter. In fact, levels of vaginal marking vary dynamically in response to odor signals conveying information about sexual identity and social status (Huck, Lisk, & Gore, 1985b; R. E. Johnston, 1977). Increases in estrogen across the first three days of the estrous cycle may instead serve to provide a slower, more gradual mechanism for increasing the overall levels of vaginal marking as the day of sexual receptivity approaches.

Despite an insignificant main effect of this study, much was learned about estrogen's effects on vaginal marking behavior. As expected, there was a statistically significant difference when vaginal marking frequency scores from the Pre-OVX condition were compared to respective scores from Post-OVX trials; in other words, vaginal marking frequency significantly decreased after ovariectomy. This finding supports the research suggesting that vaginal marking behavior is modulated by estrogen (Burnett, et al., 1985). The specific effects of ovariectomy reduced circulating estradiol—while preserving locomotion—resulting in significant reduction of vaginal marking frequency. Though unexpectedly, the reduction of vaginal marking behavior after ovariectomy persisted through all subsequent conditions. It is possible that estrogen delivered in this aforementioned dosage and route of administration simply does not rapidly affect behavior in the golden hamster; however, it is also possible that ovariectomy surgery—and subsequent removal of circulating estrogen—removed the subjects of their ability or affinity to specifically perform vaginal marking behavior despite hormone injections. Although this evidence does not discount the possibility of strict rapid control of estrogen on vaginal marking behavior, these points illustrate shortcomings of this present study because vaginal marking behavior was not replenished at any time after ovariectomy surgery. A positive control of estrogen's effect on vaginal marking after ovariectomy and the evidence of the efficacy of the hormone injections could better explain these non-significant results in future research.

Vaginal marking frequency was observed at high levels in Pre-OVX trials when intact proestrous females were exposed to male odors; however, vaginal marking frequency was variable between subjects. Ethologically, this variability is beneficial to the success of the golden hamster

species. In the wild, golden hamsters cover a large territory with many different environmental constraints between animals. Constantly vaginal marking is useful in safe and social environment; however, doing so in an unsafe environment could attract predators. Thus, vaginal marking is highly variable between animals in order to perpetuate the species. Although this variability was reduced after ovariectomy surgery and controlled for by a within-subjects experimental design, high variability may present the idea that some animals are more prone to estrogen's effects than others. In light of confounding effects of variability on main effects of this experiment, the present study's exploration of vaginal marking has enhanced the body of research describing this behavior in a laboratory setting.

Though describing the mechanism whereby estrogen affects sexual behavior is the proximal goal of this study, improving the quality of life of human patients suffering from non-existent or hyperactive sexual motivation is the ultimate goal of these experiments. Humans often experience similar circulations of estrogen described in this animal model. Endogenous estrogen levels undulate during female menstrual cycles and medical treatments for cancer and other hormone replacement issues include supplemental hormone treatments that often affect sexual motivation (Basson, 2009; Bruce & Rymer, 2009). As an exploratory study of estrogen's affect on sexual appetitive behavior, the present experiment could lead to more extensive knowledge on how sexual motivation is affected by hormone therapy. There are also possible pharmaceutical benefits that could lead to specifically affecting sexual motivation either by medical enhancement or reduction.

In light of several shortcomings that attack the validity of this present study, a second study is currently underway that aims to describe estrogen's rapid effects on vaginal marking when low (comparable to the lowest endogenous levels of intact female golden hamsters) controlled levels of estrogen are maintained with subcutaneously implanted estrogen capsules. This second study seeks to discuss how estrogen dramatically and rapidly affects sexual behavior by the activation of non-classical mechanisms from classical mechanisms of circulating hormone. The study also will entail a series of positive control estrogen assays in order to confirm the blood levels of estrogen across differing experimental conditions. These data will theoretically confirm or deny that 17β -estradiol in a CDX vehicle rapidly distributes estrogen throughout the animal's system and rapid behavioral changes occur as a result.

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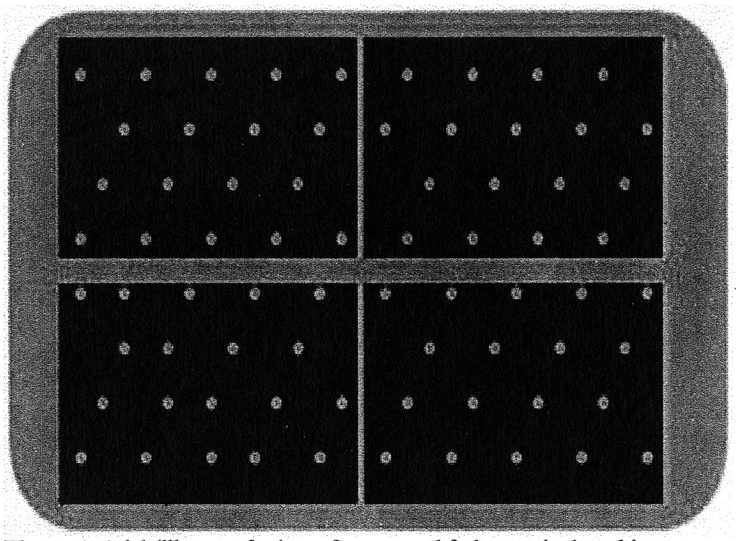

Figure 1. Arial (illustrated) view of an upward facing vaginal marking plate. Plates were lowered into stimulus cages in this orientation (top).

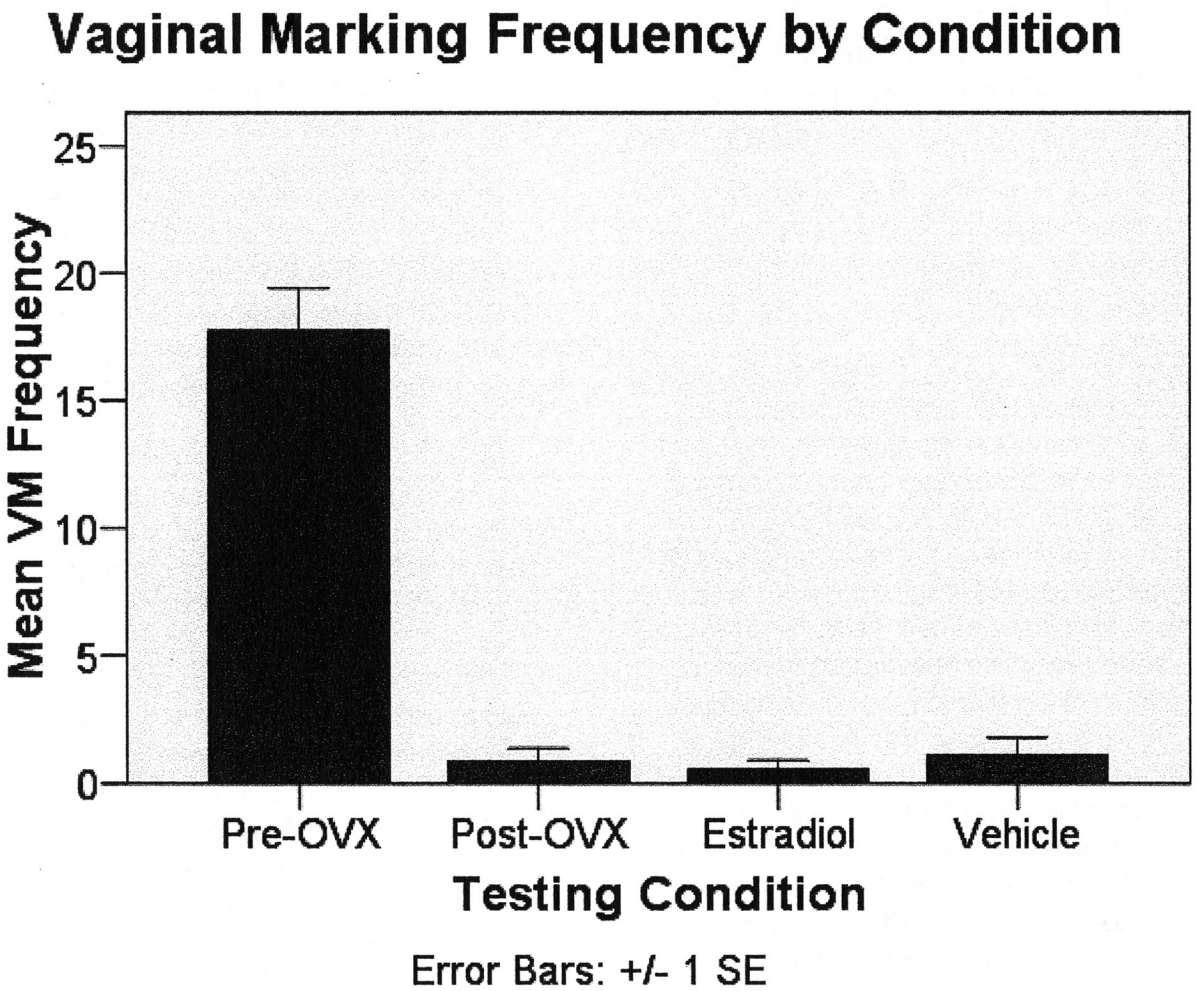

Figure 2.
Error bars: +/- 1 SE. *$p < .05$ after Bonferonni correction for multiple pairwise comparisons. Pre-OVX VM Freq was significantly different from VM Freq in every other testing condition

Table 1.
Values are mean ± SEM. After ovariectomy, there were no differences in vaginal marking frequency across conditions, $p > .05$. Furthermore, no differences in line cross scores existed across conditions $F(3,24) = 40.962, p = .06$

Summary of behavioral measures across conditions

Experimental Condition	Vaginal Marking Frequency	Line Crosses (Locomotion)
Pre-OVX	17.7 ± 5.1	29.6 ± 8.4
Post-OVX	0.9 ± 1.7	22.7 ± 8.2
Estradiol	0.6 ± 1.0	25.6 ± 7.7
Vehicle	1.1 ± 2.1	25.6 ± 5.0

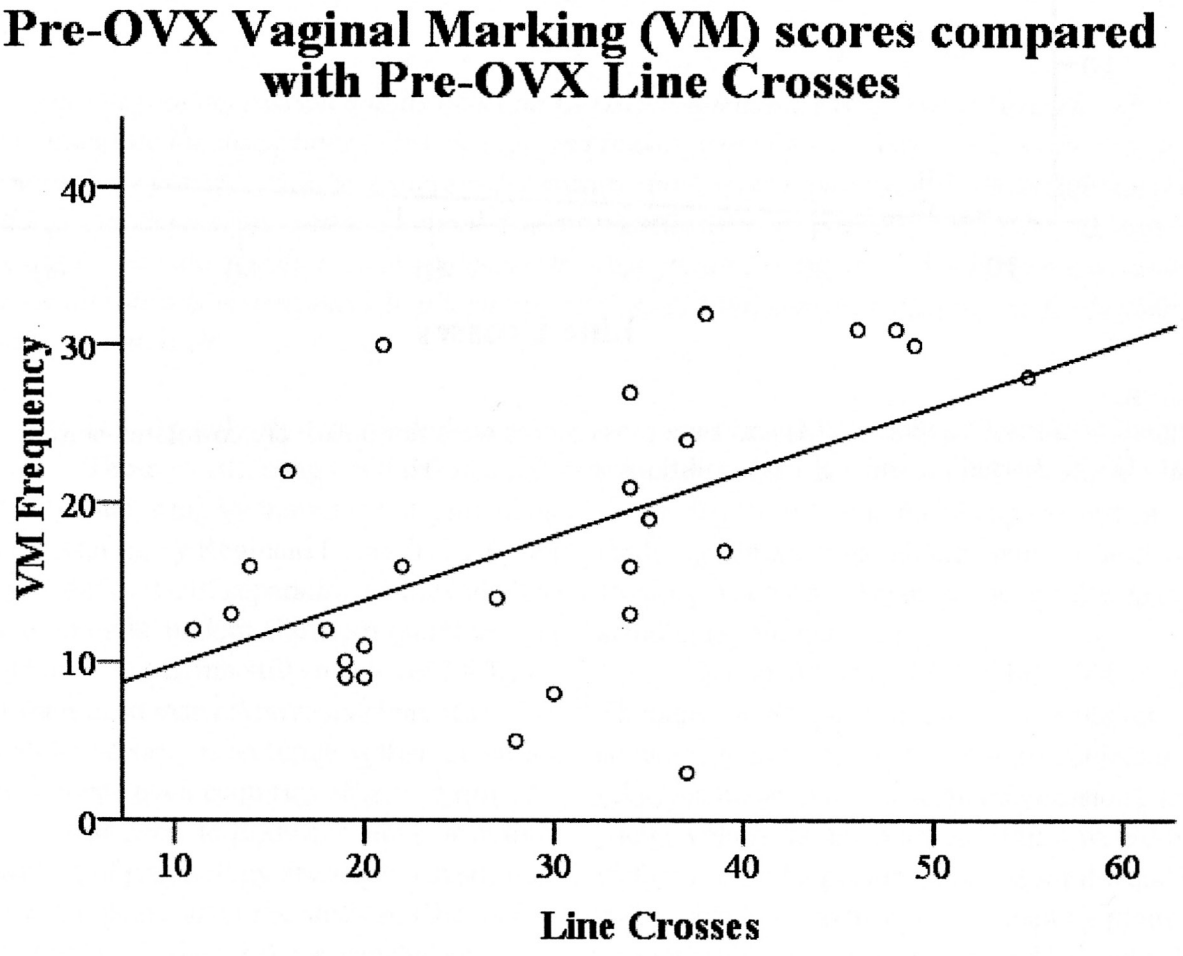

Figure 3a.
Vaginal marking frequency (VM) and Line crosses were positively correlated in Pre-OVX trials, $r = .60, p < .01$

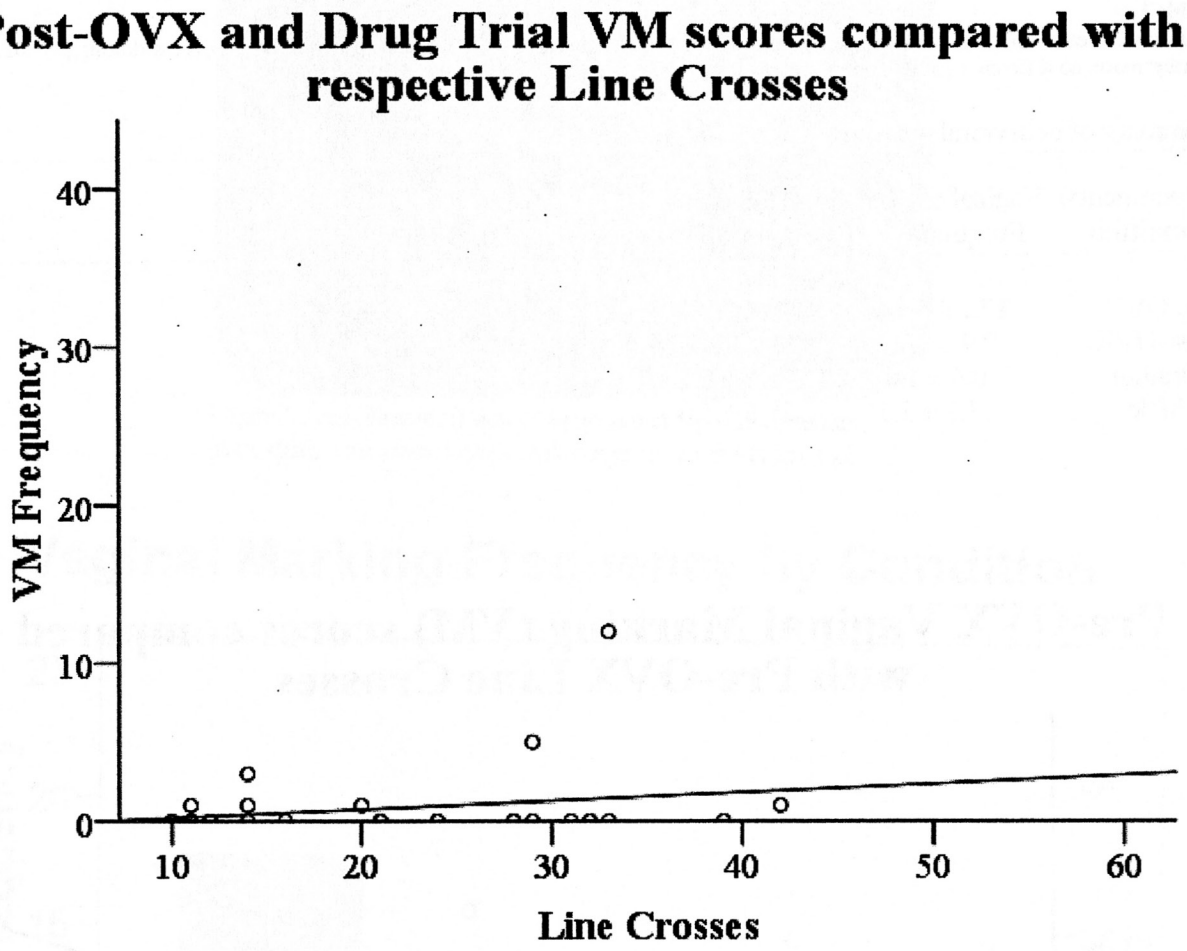

Figure 3b.
Vaginal marking frequency (VM) and Line cross scores were not positively correlated across Post - OVX , Estradiol, and Vehicle conditions, $r = .09$, $p > .05$