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The Effects of Selective Estrogenic Drugs in the Medial Amygdala on Male Rat Sexual Behavior

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THE EFFECTS OF SELECTIVE ESTROGENIC DRUGS IN THE MEDIAL AMYGDALA ON MALE
RAT SEXUAL BEHAVIOR

by

EJIROGHENE VIVIAN OGAGA-MGBONYEBI

Under the Direction of Andrew N. Clancy, PhD

ABSTRACT

Male rat copulatory behavior is dependent on Testosterone (T) and its metabolites, estradiol (E_2) and dihydrotestosterone (DHT). The estrogen receptor (ER) isoforms, $ER\alpha$ and $ER\beta$, exist in the medial Amygdala (MEA) and either receptor might mediate mating behavior. Therefore, the effects of selective estrogenic MEA implants: propyl pyrazole triol (PPT, $ER\alpha$ agonist), diarylpropionitrile (DPN, $ER\beta$ agonist), and 1-methyl-4-phenyl pyridinium (MPP, $ER\alpha$ antagonist) were compared to E_2 in maintaining sexual behavior. Four groups of male rats were castrated and administered DHT s.c. and bilateral MEA implants containing either cholesterol, E_2 , PPT or DPN. An additional group of gonadally intact male rats received bilateral MPP-MEA implants. The post-surgical trials showed a significant decrease in the mating behavior of groups that received cholesterol, PPT, or DPN-MEA implants. However, sexual behavior was maintained in male rats that received the E_2 or MPP-MEA implants. These results suggest a differential response of the MEA to E_2 .

INDEX WORDS: Estradiol, Estrogen receptor, Medial amygdala, Sexual behavior, Propyl pyrazole triol (PPT), Diarylpropionitrile (DPN), Methyl-piperidino-pyrazole (MPP), Mating

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

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Georgia State University

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This thesis is dedicated to my loving parents,
Godspower Mgbonyebi and Patience Ogaga-Mgbonyebi,
for their support, encouragement, and unwavering
confidence in my ability to succeed in all my endeavors.

I thank the Lord God for enabling me by His grace
to successfully finish this chapter of my academic journey.

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LIST OF ABBREVIATIONS

AS-ODN	antisense oligodeoxynucleotides
BSA-E ₂	estrogen conjugated bovine serum albumin
CHOL	cholesterol
DHT	dihydrotestosterone
DPN	diarylpropionitrile
E ₂	estradiol
EF	ejaculation frequency
EL	ejaculation latency
ER	estrogen receptors
ER α	estrogen receptor alpha
ER β	estrogen receptor beta
ERKO	estrogen receptor knockout
α ERKO	estrogen receptor alpha knockout
β ERKO	estrogen receptor beta knockout
EST	eastern standard time
IF	intromission frequency
MEA	medial amygdala
MF	mount frequency
ML	mount latency
MPO	medial preoptic area
MPP	methyl-piperidino-pyrazole
PEI	post-ejaculatory interval

PPT propyl pyrazole triol

s.c. subcutaneous

T testosterone

INTRODUCTION

Mammalian male sexual behavior depends on gonadal steroid hormones such as testosterone (T) and its metabolites [Christensen and Clemens, 1974; Davidson, 1969]. In the brain, testicular T is irreversibly converted into the active metabolites, dihydrotestosterone (DHT) via 5α -reductase [Massa et al., 1972] and estradiol (E_2) via aromatase [Naftolin et al., 1975]. Testosterone produced by testicular leydig cells facilitates the expression of male sexual behavior; however, the removal of the testes results in decreased sexual behavior [Davidson, 1966a, 1966b]. Exogenous T can restore mating behavior in castrated male rats [Christensen and Clemens, 1974; Davidson, 1966a, 1966b], and studies have shown that certain metabolites of T administered within physiological dose ranges can influence the expression of sexual behavior. Whereas neither exogenous E_2 [Södersten, 1973] nor exogenous DHT [Davidson, 1966a; McGinnis and Dreifuss, 1989] administered alone completely restored mating behavior, a combination of E_2 and DHT administered within the physiological dose range was as effective as T in restoring mating behavior in castrated male rats [Baum and Vreeburg, 1973]. Therefore, the androgenic and estrogenic metabolites of T are necessary for the expression of mating behavior in male rats.

Fadrozole, a non-steroid aromatase inhibitor [Lipton et al., 1990; Bonsall et al., 1992; Vagell and McGinnis, 1997], blocks the conversion of T to E_2 . When administered systemically [Bonsall et al., 1992] or locally into the medial amygdala (MEA) [Huddleston et al., 2006; Huddleston et al., 2003], Fadrozole decreases male rat mating behavior. Conversely, systemic administration of E_2 [Bonsall et al., 1992] or E_2 -MEA implants [Huddleston et al., 2003] partially reversed this decrease in mating behavior. These studies suggest that, in the MEA, estrogen is required for the expression of male rat mating behavior. Estrogenic metabolites of T act on steroid sensitive neurons that express estrogen receptors (ERs) in both the medial preoptic area (MPO) [Clancy et al., 1995, 2000] and the MEA [Huddleston et al., 2003, 2006]. Two estrogen receptor (ER) isoforms, $ER\alpha$ and $ER\beta$, exist in the MEA [Shughrue and Merchenthaler, 2001; Greco et al., 2003] and either one of these ER subtypes might mediate the action of estrogen in the MEA. ER-Knock-Out (ERKO) experiments in male mice have shown that the deactivation of $ER\alpha$ significantly reduces mating behavior, while β ERKO does not decrease mating behavior [Ogawa et al., 1997,

1998, 1999; Rissman et al., 1999]. However, in the combined knock-out, deactivation of both ER α and ER β receptors completely eliminates all sexual behaviors of the male mice [Ogawa et al., 2000] which suggest that ER α plays a dominant role in the expression of mating behavior, whereas ER β plays a minor role. Furthermore, ER α antisense oligodeoxynucleotide (AS-ODN) infusion of the MEA locally inhibited ER α expression in the MEA, but there was no significant decrease in the mating behavior of male rats [Paisley et al., 2005, Paisley, 2007]. In contrast, infusion of ER α AS-ODN to the MPO significantly decreased male rat mating behavior [Paisley, 2007; Paisley et al., 2005]. Based on these findings, we hypothesized that the MEA responds differently to E₂ than the MPO, and a different ER pathway, not ER α but perhaps ER β , might mediate the sexual response of the male rat MEA to E₂.

Using selective estrogenic drugs, we tested the hypothesis by comparing the sexual behavior of castrated male rats before and after the drug implants into the MEA. Four groups of castrated DHT s.c. maintained male rats received bilateral MEA implants containing one of the following drugs: (i) cholesterol (negative control), (ii) E₂ (positive control), (iii) Propyl pyrazole triol (PPT, ER α agonist [Stauffer et al., 2000]) or (iv) Diarylpropionitrile (DPN, ER β agonist [Meyers et al., 2001]). Additional gonadally intact male rats received bilateral MEA implants containing Methyl-piperidino-pyrazole (MPP, ER α antagonist [Sun et al., 2002]). We predicted that in the MEA, PPT, an ER α agonist would not maintain mating behavior as compared to E₂, but DPN, an ER β agonist would promote a better sexual response than PPT when compared to E₂. In addition, we expected the group receiving MPP (ER α antagonist) implants to continue mating because previous studies have shown a greater role of ER α in the MPO than the MEA.

MATERIALS AND METHODS

Animals

Male and female Sprague Dawley rats obtained from Charles River Laboratories were housed separately in polycarbonate cages, 22 x 44 x 18 cm, with free access to food and water at Georgia State University. The rats were kept in a climate-controlled rat colony under a 14:10 hour reverse light-dark cycle (lights off at 0930h EST). Male rats were housed two per cage until surgery after which they were housed individually and females remained housed two per cage throughout the experiment. All maintenance and surgical procedures were in accordance with Georgia State University IACUC and the NIH Guide for the Care and Use of Laboratory Animals (NIH Publ. No. 85-23, revised 1985).

Female rats were anesthetized with isoflurane gas in a chamber (5% gas at 10 LPM oxygen exchange rate) and transferred to a nosecone (2-3% gas at 3 LPM oxygen). Females were ovariectomized through a single midline abdominal incision and implanted subcutaneously (s.c.) with a 6 mm Silastic capsule filled with crystalline E₂. Postoperatively, the females received Penicillin (0.1 ml in benzathine penicillin G+ procaine penicillin G) to alleviate surgical pain and reduce the possibility of infection. The recovery time for the females was seven days before behavioral testing. They were injected with 1.0 mg s.c. progesterone (1 mg progesterone/0.2 ml sesame oil) 4-6 hours before being tested with males.

In four weekly 30-minute mating tests, male rats were paired with receptive females and screened for sexual behavior for inclusion in the experiment. Proven ejaculators were matched based on total ejaculatory frequency and assigned to four groups: (i) E₂-MEA (positive control), (ii) Cholesterol-MEA (negative control), (iii) PPT-MEA, and (iv) DPN-MEA. In addition, a fifth group consisting of gonadally intact male rats received MPP-MEA implants during postoperative trials.

Male Sexual Behavior

Male rats were paired with sexually receptive females during preoperative and postoperative mating tests for 30 minute in a 22 x 44 x 50 cm testing arena under dim red light. The behavioral testing began three hour after light off. Observers blind to the study recorded the following behaviors: mount fre-

quency (MF- the number of mounts without penetration), intromission frequency (IF-the number of mounts with penetration), and ejaculation frequency (EF-the number of ejaculations). Observers also recorded the mount latency (ML - time from the start of the test until the first mount or intromission or 1800 seconds if no mount occurred), ejaculation latency (EL - time from the first mount or intromission until the first ejaculation or 1800 seconds if no ejaculation occurred), and post-ejaculatory interval (PEI - time between an ejaculation and the next mount or intromission or 1800 seconds if no ejaculation occurred).

Administration of Selective Estrogenic Drugs into the MEA

Groups of proven ejaculators were anesthetized with isoflurane gas and castrated through a single midline abdominal incision and implanted subcutaneously (s.c.) with a 10 mm Silastic capsule (1.981 mm ID x 3.175 mm OD) filled with crystalline DHT. Such DHT capsules have been reported to produce circulating DHT levels in the physiological range [Ando et al., 1998; Lugg et al., 1995; Parte and Juneja, 1992]. Immediately after castration and administering subcutaneous DHT capsules, the male rats were placed in a stereotaxic instrument and implanted bilaterally with 22-gauge stainless steel, ethylene oxide sterilized guide cannulae aimed at the MEA (level skull coordinates: AP = -3.2 mm, ML = 3.5 mm, DV = -8.2 mm [Swanson, 1998]). Drug-carrying 28-gauge sterile, stainless steel, inner cannulae were inserted through the guides and their tips extended 1 mm below the guide cannulae. These were tamped in either, E₂, cholesterol, PPT, or DPN, sterilized, and inserted into the guide cannulae. A fifth group of gonadally intact male rats received MPP-MEA implants using the above stereotaxic procedure. All animals were given five to seven days to recover before weekly postoperative behavioral testing resumed. 48 hours before and 24 hours after every behavioral test, freshly tamped and sterilized drug-delivery inner cannulae were inserted into the brain under brief isoflurane anesthesia. Cannulae tips were inspected microscopically to ensure continuous brain exposure to the appropriate drug. When cannulae from the experimental animals were inspected, the drug was present at the cannulae tips at least 98% of the time.

Histological Verification

After the postoperative behavioral tests, male rats were euthanized with an overdose of sodium pentobarbital (100 mg/kg i.p.) and perfused transcardially with physiological saline for five minutes followed by a minimum of 300ml of a formalin fixative solution (pH 7.4). Skulls were partially opened and immersed overnight in perfusion fixative. Brains were removed from skulls the next day and stored in 30% sucrose in 0.1 M PB for at least 48 hours. Frozen coronal brain sections (40 μ m) through the diencephalon were collected into 0.1 M PB, mounted on gel-albumin-coated slides, and stained with toluidine blue to verify cannulae placement.

Statistical Analysis

We analyzed between-group behavioral data from the pre-surgical period and separately during the post-surgical period using a one-way ANOVA analyses of variance, followed by *post hoc* comparisons using the Fisher least significant difference test at a probability level of 0.05 [Kirk, 1968]. Within-group comparisons of pre-surgical and post-surgical changes in behavior were analyzed by paired t-tests [Kirk, 1968]. Data from all males were used in the statistical analyses; default values (*see sexual behavior tests*) were used in situations where animals did not display a given behavior. Two-tailed probabilities are reported in all cases.

RESULTS

Male Sexual Behavior

After four to seven weeks of postoperative testing, the between group differences before and after surgery and the within group differences in sexual performance were determined by using the terminal pre-surgical sexual behavioral scores and the terminal post-surgical sexual behavioral scores. During the pre-surgical trials, all four groups mated robustly with no significant group difference in any of the behavioral indices. However, after surgery, groups administered PPT, DPN, or cholesterol-MEA implants mated significantly less frequently than the E₂-MEA group which continued to mount vigorously (FIGURE 1). Significant group differences emerged in mount frequency ($F_{3, 24} = 6.04$, $P < 0.003$), intromission frequency ($F_{3, 24} = 4.43$, $P < 0.013$), and mount latency ($F_{3, 24} = 5.12$, $P < 0.007$) after surgery. Specifically, the between group *post hoc* analysis showed that after surgery the E₂-MEA group mounted ($P < 0.05$) and intromitted significantly ($P < 0.05$) more often than the other three groups, and the E₂-MEA group also had a significantly shorter ($P < 0.05$) mount latency than the DPN and cholesterol groups. This *post hoc* analysis of the E₂, PPT, DPN and cholesterol groups also showed no significant group differences in ejaculation frequency, ejaculation latency, or post-ejaculatory interval. Thus, E₂-MEA implants in castrated, DHT s.c. maintained male rats supported mounting, whereas, the PPT, DPN, and cholesterol MEA implants did not maintain mounting, intromission, or ejaculation.

Subsequently, within group differences in mating behavior were examined before and after surgery. In the E₂-MEA group, there were no significant changes in mount frequency, mount latency or the post-ejaculatory interval before and after surgery; however, the intromission frequency ($t = 2.49$, $P < 0.047$) and ejaculation frequency ($t = 2.49$, $P < 0.047$) were significantly lower after surgery, and the ejaculation latency was significantly longer after surgery. The PPT-MEA group declined significantly in mount frequency ($t = 2.55$, $P < 0.043$) and intromission frequency ($t = 4.32$, $P < 0.005$), whereas, mount latency ($t = 2.77$, $P < 0.032$) and post-ejaculatory interval ($t = 2.73$, $P < 0.034$) were significantly longer during the post-surgical trials. In the DPN-MEA group, mount frequency ($t = 2.73$, $P < 0.034$), ejaculation

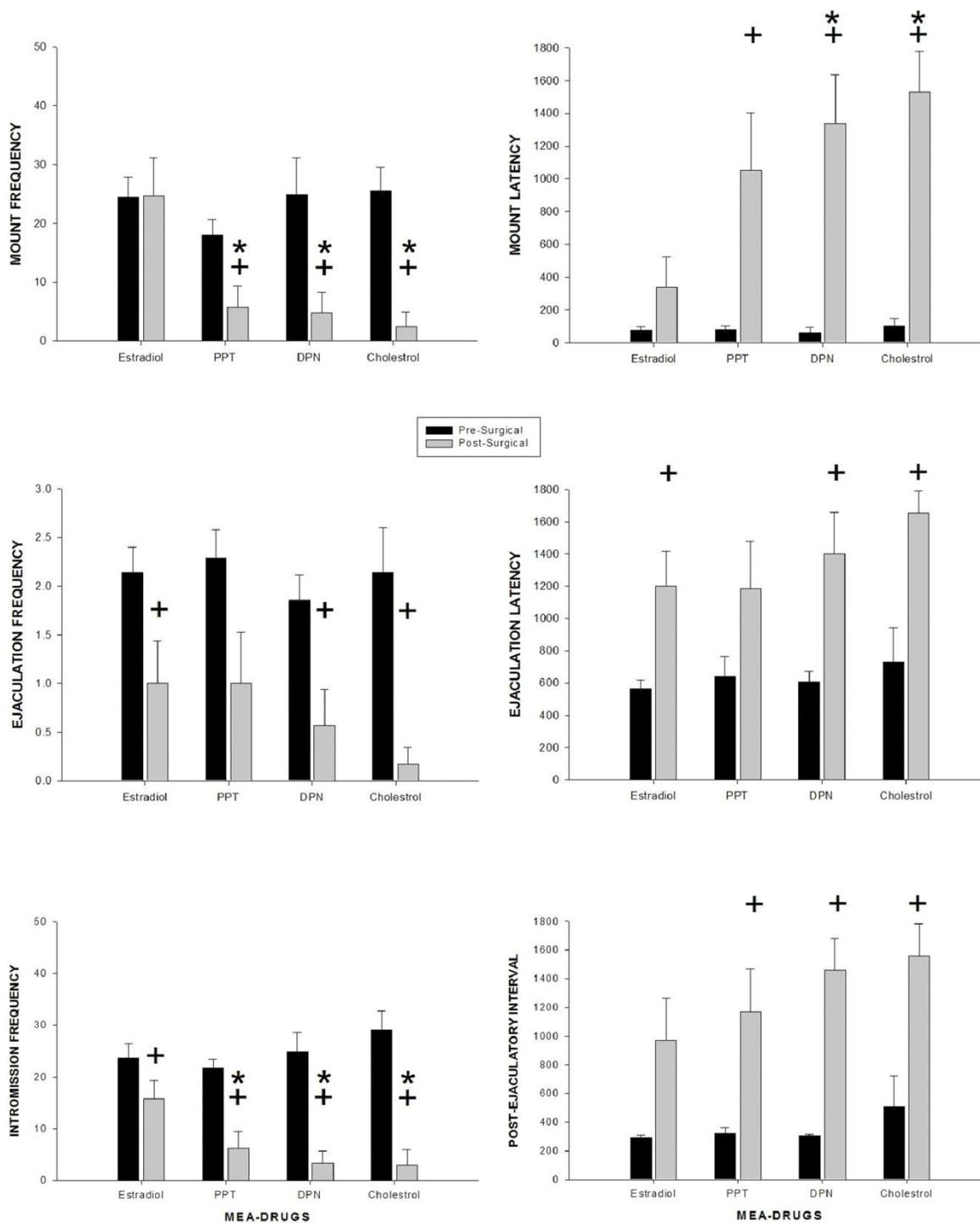


FIGURE 1: MALE SEXUAL BEHAVIOR. Pre-surgical and post-surgical mating behaviors (MEAN \pm standard error) of all groups. Castrated male rats given either PPT, DPN or cholesterol MEA implants mated significantly less than male rats that received E₂-MEA implants during post-surgical trials. Asterisks (*) indicate between group difference from estrogen ($p < 0.05$). Cross symbol (+) indicates behaviors in which within group comparison of pre-surgical trials and terminal post-surgical trials differed significantly ($p < 0.05$).

frequency ($t = 4.5$, $P < 0.004$) and intromission frequency ($t = 5.23$, $P < 0.002$) significantly decreased during the post-surgical trials, whereas the mount latency ($t = 4.25$, $P < 0.005$), ejaculation latency ($t = 3.26$, $P < 0.017$) and post ejaculatory interval ($t = 5.38$, $P < 0.002$) significantly increased. Similarly, the cholesterol-MEA group showed a significant decline in mount frequency ($t = 5.05$, $P < 0.002$), ejaculation frequency ($t = 4.58$, $P < 0.004$) and intromission frequency ($t = 7.72$, $P < 0.000$) during post-surgical trials, while mount latency ($t = 14.4$, $P < 0.000$), ejaculation latency ($t = 3.96$, $P < 0.007$), and post-ejaculatory interval ($t = 3.95$, $P < 0.008$) became significantly longer. These results indicate that in contrast to E₂-MEA implants which maintained mount frequency and mount latency, the MEA implants of PPT, DPN, or cholesterol alone did not maintain mating behavior in castrated male rats receiving DHT s.c.

Finally, the terminal (week 4) pre-surgical mating behavior of the MPP-MEA group did not differ significantly from the terminal (week 5) post-surgical mating behavior in mount plus intromission frequency (pre-surgical Mean \pm SEM = 53.29 ± 2.73 versus post-surgical Mean \pm SEM = 47.14 ± 6.43 , N = 7, paired t-test = 1.24, $p < 0.26$, n.s.) or ejaculation frequency (pre-surgical Mean \pm SEM = 2.14 ± 0.26 versus post-surgical Mean \pm SEM = 2.57 ± 0.48 , N = 7, paired t-test = 1.16, $p < 0.29$, n.s.), which indicates that MPP (an ER α antagonist) did not inhibit mating behavior following implantation into the MEA of gonadally intact male rats.

Cannula Location

The locations of cannulae tips within the MEA are shown in FIGURE 2. Cannulae tips were located in or near the MEA in all groups of animals.

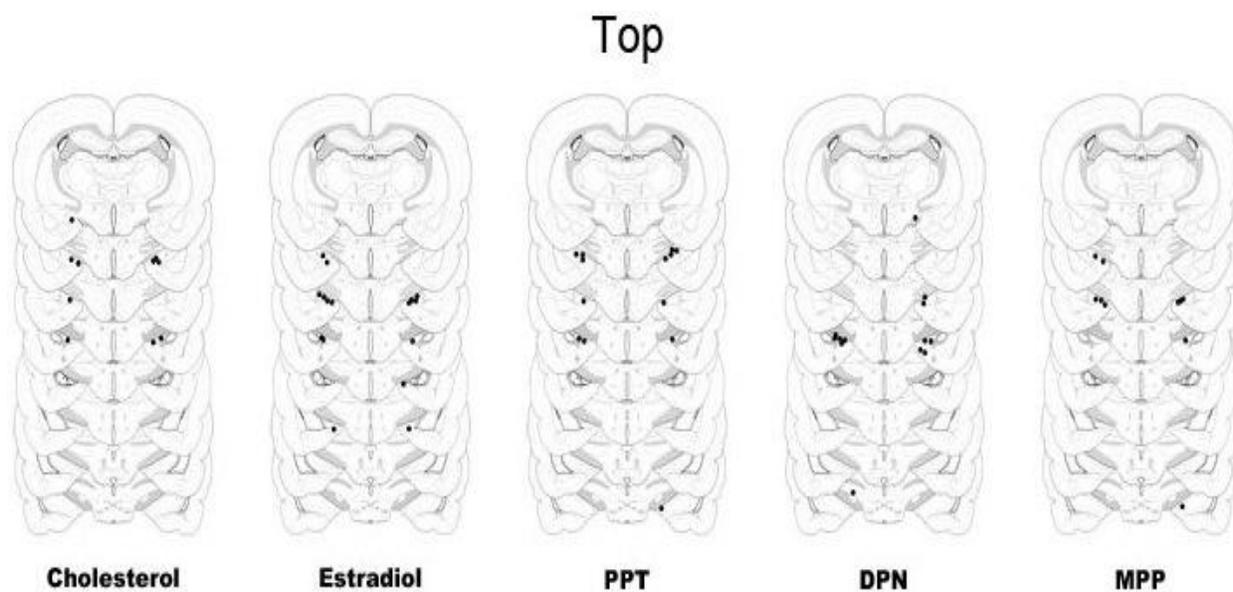


Figure 2

FIGURE 2: CANNULA LOCATION IN THE MEA. Cannulae tips were positioned in or near the MEA in all groups. The black dots represent the locations of the cannulae tips.

DISCUSSION

During the post-surgical period, the mating behavior of the castrated male rats receiving DHT s.c. with either PPT (ER α agonist), DPN (ER β agonist), or cholesterol (negative control) MEA implants significantly decreased compared to those receiving E₂ to the MEA. Pre-surgical versus post-surgical sexual behavior comparison revealed that animals implanted in the MEA with either PPT, DPN, or cholesterol had significant reductions in the number of mounts, intromissions and ejaculations. In contrast, male rats with the E₂-MEA implants continued to mount robustly at levels that were not significantly different than the pre-surgical period. In addition, gonadally intact male rats receiving MPP-MEA implants continued to mate after surgery, and the pre-surgical and post-surgical frequencies of mounting, intromission, and ejaculation did not differ significantly.

The purpose of this study was to test the hypothesis that the MEA responds differently to E₂ than the MPO (in which ER α mediates the sexual response to E₂ [Russell, 2010]), whereas, a different ER pathway, not ER α , but possibly ER β might mediate the sexual response of the MEA to E₂. Therefore, the behavioral effects of selective estrogenic MEA implants of PPT, DPN, MPP or cholesterol (negative control) were compared to the effects of E₂ implants in the MEA. We predicted that DPN (ER β agonist) implants in the MEA would maintain mating behavior, whereas, PPT-MEA (ER α agonist) implants would not maintain the behavior. We also expected that MPP (ER α antagonist) would not interfere with mating behavior because a previous study showed that knock-down of ER α in the MEA did not reduce mating in male rats [Paisley, 2007]. Our hypothesis was partially supported. The results showed a significant decrease in the number of mounts and/or intromissions in all groups except those receiving E₂ or MPP MEA implants which replicate previous findings that E₂ in the MEA promotes mounting but not ejaculation [Huddleston et al., 2003]. However, our current data suggests that, in the MEA, neither ER α nor ER β alone is sufficient for the expression of mating behavior in male rats.

E₂ and synthetic ligands such as PPT, DPN, and MPP selectively bind different ER subtypes and elicit different responses [Stauffer et al., 2000]. In the MEA, E₂ activates both ER α and ER β and promotes male sexual behavior. PPT has a greater binding affinity for ER α and limited cross reactivity with

ER β [Stauffer et al., 2000], whereas DPN strongly binds ER β with little cross reactivity with ER α [Meyers et al., 2001]. Cholesterol is presumed to be inert and does not activate either ER α or ER β , and as expected, the group that received cholesterol implants to the MEA ceased mating (FIGURE 1). MPP, an ER α antagonist, has a high binding affinity for ER α and little cross reactivity with ER β [Sun et al., 2002], and as expected, it did not decrease mating behavior when implanted into the MEA. The outcome of these drugs in the MEA suggests that E₂ indeed acts differently in the MEA than in the MPO.

The conversion of T to DHT by 5-alpha reductase [Massa et al., 1972] and of T to E₂ by aromatase is vital for the expression of sexual behavior in many male mammals [Naftolin et al., 1975; Whalen et al., 1985]. In castrated male rats, mating behavior is restored by exogenous T [Christensen and Clemens, 1974; Davidson, 1966a, 1966b] or by combined treatment with DHT and E₂ [Baum and Vreeburg, 1973]. Moreover, the MEA is a critical brain region for the display of sexual behavior because bilateral lesions of the MEA cause severe deficits in male rat copulatory behavior [Kondo, 1992; Harris and Sachs, 1975]. The MEA receives chemosensory inputs from the olfactory and accessory olfactory systems [Coolen et al., 1997; Canteras et al., 1995; Wood and Newman, 1995] along with somatosensory inputs from the genitals [Baum and Everitt, 1992; Gréco et al., 1999] to regulate male sexual behavior. The male rat MEA contains both androgen and estrogen sensitive neurons that are activated by mating [Gréco et al., 1998]. Blocking the aromatization of T to E₂ within the MEA via the infusion of fadrozole (an aromatase inhibitor) reduces mating [Huddleston et al., 2006; Huddleston et al., 2003] while E₂ reverses this action [Huddleston et al., 2006]. In castrated male rats, E₂-MEA implants restore mounting behavior alone [Rasia-Filho et al., 1991] while in male hamsters, E₂-MEA implants increase mounting and noncopulatory behaviors such as interaction with females, self-grooming, and ano-genital investigation [Wood, 1996]. Likewise, Huddleston et al., [2003] reported that E₂ in the MEA facilitates mounting but not ejaculation. Our current data replicate and extend these previous studies by showing that E₂ in the MEA supports mounting (FIGURE 1) which reflects sexual motivation in male rats. Although E₂ in the MEA promotes mounting in castrated male rats, the MPO responds differently to E₂. Lesions of the MPO result in the complete elimination of male rat sexual behavior [Liu et al., 1997b] while E₂ implants in the MPO of

male rats maintains all components of rodent sexual behavior (mounting, intromission, and ejaculation) [Clancy et al., 2000]. The MEA appears to play a role in sexual motivation by integrating chemosensory inputs from the olfactory systems, somatosensory and other sensory stimuli with hormonal pathways during mating [Baum and Everitt, 1992; Canteras et al., 1995; Wood and Newman, 1995; Gréco et al. 1999].

The MEA contains AR, ER, or AR colocalized with ER [Greco et al., 1998; Wood and Newman, 1995] and ER exists in two isoforms, ER α and ER β [Kuiper et al., 1996]. Estrogen sensitive neurons in the MEA have been shown to co-express ER α protein and ER β mRNA [Shughrue et al., 1998]. ER-Knock-Out (ERKO) studies in male mice showed that the deactivation of ER α significantly reduces mating behavior, while ER β KO does not greatly decrease mating behavior [Ogawa et al., 1997, 1998, 1999; Rissman et al., 1999]. Nevertheless, the combined knock-out of both ER α and ER β receptors completely eliminates mating in male mice [Ogawa et al., 2000] which suggests a critical role for ER α and a minor role for ER β in the expression of sexual behavior. Moreover, the inhibition of ER α expression in the MEA via AS-ODN infusion did not significantly affect mating in male rats [Paisley, 2007]. These previous findings together with our current results imply that neither ER α /ER α homodimerization nor ER β /ER β homodimerization in the MEA is necessary or sufficient for sexual behavior, but a combined ER α /ER β related signal transduction pathway might mediate the sexual response of the MEA to E₂; however, the mechanism by which E₂ acts in the MEA is still unknown. Our present study does not address estrogen's mechanism of action in the MEA, but Huddleston et al. [2006] suggests an intracellular mechanism of E₂ action in the MEA. To determine if E₂ mediates mating via the traditional pathway after binding intracellular receptors [Jensen et al., 1968; King and Green, 1984] or via binding to plasma membrane ER [Pietras and Szego, 1977; Minami et al., 1990], the MEA of castrated, DHT maintained male rats was chronically administered bilateral implants of E₂ conjugated to bovine serum albumin (BSA-E₂) [Huddleston et al. 2006]. BSA is a large protein that does not cross the plasma membrane. Therefore, the complex of E₂ and BSA restricts the action of E₂ to cell surface signaling by blocking E₂ diffusion across the plasma membrane [Pappas et al., 1995]. Huddleston et al. [2006] showed that chronic delivery of BSA-E₂ to the MEA does not maintain copulatory behavior, whereas free E₂ administered to the MEA

promotes mounting in male rats; this suggests that E_2 acts intracellularly within the MEA rather than at the plasma membrane. In contrast, E_2 mediates copulatory behavior via plasma membrane bound ER in the MPO, specifically $ER\alpha$ [Huddleston et al., 2006; Russell 2010].

In conclusion, we expected that $ER\beta$ would mediate the sexual response of the MEA to E_2 , but E_2 did not facilitate mating via the $ER\alpha$ or $ER\beta$ pathway alone. The findings of this study led us to believe that E_2 in the MEA is necessary for sexual motivation (as reflected by mounting) but not ejaculatory performance, whereas other studies suggest that estrogen mediates ejaculatory performance by acting in the MPO not the MEA. Potentially, no single E_2 receptor pathway in the MEA is necessary for the expression of male rat sexual behavior, but several different ER subtypes might work together in an additive fashion to regulate sexual motivation.

REFERENCES

- Baum MJ, Everitt BJ (1992) Increased expression of c-fos in the medial preoptic area after mating in male rats: role of afferent inputs from the medial amygdala and midbrain central tegmental field. *Neuroscience* 50:627-646.
- Baum MJ, Vreeburg JTM (1973) Copulation in castrated male rats following combined treatment with estradiol and dihydrotestosterone. *Science* 182(109):283-285.
- Bakker J, Honda S, Harada N, Balthazart J (2004) Restoration of male sexual behavior by adult exogenous estrogens in male aromatase knockout mice. *Horm Behav* 46:1-10.
- Beach FA, Holz-Tucker AM (1949) Effects of different concentrations of androgen upon sexual behavior in castrated male rats. *J Comp Physiol. Psychol.* 42:433-453.
- Bonsall RW, Clancy AN, Michael RP (1992) Effects of the nonsteroidal aromatase inhibitor, Fadrozole, on sexual behavior in male rats. *Horm Behav* 26:240-254.
- Canteras NS, Simerly RB, Swanson LW (1995) Organization of projections from the medial nucleus of the amygdala: a PHAL study in the rat. *J Comp Neurol* 260:213-245.
- Christensen LW, Clemens LG (1974) Intrahypothalamic implants of testosterone or estradiol and resumption of masculine sexual behavior in long-term castrated male rats. *Endocrinology* 95(4):984-990.
- Clancy AN, Zumpe D, Michael RP (1995) Intracerebral infusion of an aromatase inhibitor, sexual behavior and brain estrogen receptor-like immunoreactivity in intact male rats. *Neuroendocrinology* 61:98-111.
- Clancy AN, Zumpe D, Michael RP (2000) Estrogen in the medial preoptic area of male rats facilitates copulatory behavior. *Horm Behav* 38:86-93.
- Coolen LM, Peters HJPW, Veening JG (1997) Distribution of Fos immunoreactivity following mating versus anogenital investigation in the male rat brain. *Neuroscience* 77:1151-1161.
- Cowley SM, Hoare S, Mosselman S, Parker MG (1997) Estrogen receptors α and β form heterodimers on DNA. *J Biol Chem* 272(32):19858-19862.
- Davidson JM (1966a) Activation of the male rat's sexual behavior by intracerebral implantation of androgen. *Endocrinology* 79(4):783-794.
- Davidson JM (1966b) Characteristics of sexual behaviour in male rats following castration. *Anim Behav* 14:266-272.
- Davidson JM (1969) Effects of estrogen on the sexual behavior of male rats. *Endocrinology* 84(6):1365-1372.
- Gréco B, Blasberg ME, Kosinski EC, Blaustein JD (2003) Response of ER α -ir and ER β -ir cells in the forebrain of female rats to mating stimuli. *Horm Behav* 43:444-453.

- Gréco B, Edwards DA, Michael RP, Zumpe D, Clancy AN (1999) Colocalization of androgen receptors and mating-induced Fos immunoreactivity in neurons that project to the central tegmental field in male rats. *J Comp Neurol* 408:220-236.
- Gréco B, Edwards DA, Michael RP, Clancy AN (1998) Androgen receptors and estrogen receptors are colocalized in male rat hypothalamic and limbic neurons that express fos immunoreactivity induced by mating. *Neuroendocrinology* 67:18-28.
- Harris VS, Sachs BD (1975) Copulatory behavior in male rats following amygdaloid lesions. *Brain Res* 86:514-518.
- Huddleston GG, Michael RP, Zumpe D, Clancy AN (2003) Estradiol in the male rat amygdala facilitates mounting but not ejaculation. *Physiol Behav* 79:239-246.
- Huddleston GG, Paisley JC, Clancy AN (2006) Effects of estrogen in the male rat medial amygdala: infusion of an aromatase inhibitor lowers mating and bovine serum albumin conjugated estradiol implants do not promote mating. *Neuroendocrinology* 83:106-116.
- Jensen EV, Suzuki T, Kawashima T, Stumpf WE, Jungblut PW, DeSombre ER (1968) A two-step mechanism for the interaction of estradiol with rat uterus. *Proc Natl Acad Sci USA* 59:632-638.
- Jun S, Ying RH, William RH, Shubin S, John AK, Benita SK (2002) Antagonists Selective for Estrogen Receptor alpha. *Endocrinology* 143 (3), 941-947.
- King WJ, Greene GL (1984) Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells. *Nature* 307:745-747.
- Kirk RE (1968) Experimental design: Procedures for the behavioral sciences. Belmont, CA:Wadsworth Co.
- Kondo Y. (1992) Lesions of the medial amygdale produce severe impairment of copulatory behavior in sexually inexperienced male rats. *Physiol. Behav.* 51:939-43
- Kuiper GG, Enmark F, Peltö-Huikko M, Nilsson S, Gustafsson JA (1996) Cloning of a novel receptor expressed in the rat prostate and ovary. *Proc Natl Acad Sci USA* 93:5925-5930.
- Lipton A, Harvey HA, Demers LM, Hanagan JR, Mulagha MT, Kochak GM, Fitzsimmons S, Sander SI, Santen RJ (1990) A phase I trial of CGS 16949A: A new aromatase inhibitor. *Cancer* 65, 1279-1285.
- Liu YC, Salamone JD, Sachs BD (1997b) Lesions in medial preoptic area and bed nucleus of stria terminalis: differential effects on copulatory behavior and noncontact erection in male rats. *J. Neurosci.* 17:5245-5253.
- Massa R, Stupnicka E, Kniewald Z, Martini L (1972) The transformation of testosterone into dihydrotestosterone by the brain and the anterior pituitary. *J Steroid Biochem* 3:385-399.
- Meisel RL, Sachs BD (1994) The physiology of male sexual behavior. The *physiology of reproduction* (Knobil E, Neill JD, eds), pp 3-105. New York: Raven Press Ltd.

- Meyers MJ, Sun J, Carlson KE, Marriner GA, Katzenellenbogen BS, Katzenellenbogen JA (2001) Estrogen receptor- α potency-selective ligands: structure-activity relationship studies of diarylpropionitriles and their acetylene and polar analogues. *J Med Chem* 44:4230–4251
- McGinnis MY, Dreifuss RM (1989) Evidence for a role of testosterone-androgen receptor interactions in mediating masculine sexual behavior in male rats. *Endocrinology* 124(2):618-626.
- Minami T, Oomura Y, Nabekura J, Fukuda A (1990) 17β -Estradiol depolarization of hypothalamic neurons is mediated by cyclic AMP. *Brain Res.* 519:301–307.
- Naftolin F, Ryan KJ, Davies IJ, Reddy VV, White RJ, Takaoka Y, Wolin L (1975) The formation of estrogens by central neuroendocrine tissues. *Recent Prog Horm Res* 31:295-319.
- Ogawa S, Chan J, Chester AE, Gustafsson J-Å, Korach KS, Pfaff DW (1999) Survival of reproductive behaviors in estrogen receptor β gene-deficient (β ERKO) male and female mice. *Proc Natl Acad Sci USA* 96(22):12887-12892.
- Ogawa S, Chester AE, Hewitt SC, Walker VR, Gustafsson J-Å, Smithies O, Korach KS, Pfaff DW (2000) Abolition of male sexual behaviors in mice lacking estrogen receptors α and β ($\alpha\beta$ ERKO). *Proc Natl Acad Sci U S A* 97(26):14737-14741.
- Ogawa S, Lubahn DB, Korach KS, Pfaff DW (1997) Behavioral effects of estrogen receptor gene disruption in male mice. *Proc Natl Acad Sci U S A* 94:1476-1481.
- Ogawa S, Washburn TF, Taylor J, Lubahn DB, Korach KS, Pfaff DW (1998) Modifications of testosterone-dependent behaviors by estrogen receptor- α gene disruption in male mice. *Endocrinology* 139(12):5058-5069.
- Pappas TC, Bahiru G, Watson C (1995) Membrane estrogen receptor identified by multiple antibody labeling and impeded-ligand binding. *J. FASEB* 9:404–410.
- Pietras R, Szego CM (1977) Specific binding sites for oestrogen at the outer surfaces of isolated endometrial cells. *Nature* 265:69–72.
- Paisley JC, Huddleston GG, Denman HN, Carruth LL, Grober MS, Petrulis A, Clancy AN (2005). Inhibition of estrogen receptor synthesis in the medial preoptic area, but not the medial amygdala, reduces male rat mating behavior. *Horm. Behav.* 48, 94.
- Paisley JC (2007) "Differential Effects of Estrogen Receptor alpha Suppression by Antisense Oligodeoxynucleotides in the Medial Preoptic Area and the Medial Amygdala on Male Rat Mating Behavior" *Biology Theses*. Paper 12. http://digitalarchive.gsu.edu/biology_theses/12
- Rasia-Filho AA, Peres TMS, Cubilla-Gutierrez FH, Lucion AB (1991) Effect of estradiol implanted in the corticomедial amygdala on the sexual behavior of castrated male rats. *Braz J Med Biol Res* 24:1041– 9.
- Rissman EF, Wersinger SR, Fugger HN, Foster TC (1999) Sex with knockout models: behavioral studies of estrogen receptor α . *Brain Res* 835:80-90.
- Rissman EF, Wersinger SR, Taylor JA, Lubahn DB (1997) Estrogen receptor function as revealed by knockout studies: neuroendocrine and behavioral aspects. *Horm Behav* 31:232-243.

Russell N (2010) "ESTROGEN RECEPTOR ALPHA IN THE MEDIAL PREOPTIC AREA MEDIATES MALE RAT SEXUAL RESPONSES TO ESTROGEN" *Biology Theses*. Paper 25.

http://digitalarchive.gsu.edu/biology_theses/25

Sagar SM, Sharp FR, Curran T. (1988) Expression of c-fos protein in brain: metabolic mapping at the cellular level. *Science*. Jun 3;240 (4857):1328-31.

Shughrue PJ, Merchenthaler I (2001) Distribution of estrogen receptor β immunoreactivity in the rat central nervous system. *J Comp Neurol* 436:64-81.

Shughrue PJ, Scrimo PJ, Merchenthaler I (1998) Evidence for the colocalization of estrogen receptor beta mRNA and estrogen receptor alpha immunoreactivity in neurons of the rat forebrain. *Endocrinology* 436:64-81.

Shughrue PJ, Lane MV, Merchenthaler I (1997) Comparative distribution of estrogen receptor- α and $-\beta$ mRNA in the rat central nervous system. *J Comp Neurol* 388:507-525.

Södersten P (1973) Estrogen-activated sexual behavior in male rats. *Horm Behav* 4:247-256.

Swanson LW (1998) *Brain Maps: structure of the rat brain*, 2nd ed. Amsterdam, The Netherlands: Elsevier.

Stauffer SR, Coletta CJ, Tedesco R, Nishiguchi G, Carlson K, Sun J, Katzenellenbogen BS, Katzenellenbogen JA. (2000) Pyrazole ligands: structure-affinity/activity relationships and estrogen receptor-alpha-selective agonists. *J Med Chem*. 43(26):4934-47.

Vagell ME, McGinnis MY (1997) The role of aromatization in the restoration of male rat reproductive behavior. *J Neuroendocrinol* 9:415-421.

Whalen RE, Yahr P, Luttge WG. (1985) The role of metabolism in hormonal control of sexual behavior. In: Alder N, Pfaff D, Goy RW, editors. *Handbook of behavioral neurobiology* New York: Plenum p. 609-63.

Wood RI (1996) Estradiol, but not dihydrotestosterone, in the MEA facilitates male hamster sex behavior. *Physiol. Behav.* 59:833-841.

Wood RI, Newman SW (1995) The medial amygdaloid nucleus and medial pre-optic area mediate steroidal control of sexual behavior in the male Syrian hamster. *Horm Behav* 29:338- 53.