

Georgia State University

ScholarWorks @ Georgia State University

Biology Theses

Department of Biology

5-2-2007

Implant of a Selective Estrogen Receptor Alpha Agonist to the Male Rat Medial Preoptic Area Maintains Mating Behavior

Biniyam Seged Habteab

Follow this and additional works at: https://scholarworks.gsu.edu/biology_theses

Recommended Citation

Habteab, Biniyam Seged, "Implant of a Selective Estrogen Receptor Alpha Agonist to the Male Rat Medial Preoptic Area Maintains Mating Behavior." Thesis, Georgia State University, 2007.
doi: <https://doi.org/10.57709/1059204>

This Thesis is brought to you for free and open access by the Department of Biology at ScholarWorks @ Georgia State University. It has been accepted for inclusion in Biology Theses by an authorized administrator of ScholarWorks @ Georgia State University. For more information, please contact scholarworks@gsu.edu.

**IMPLANT OF A SELECTIVE ESTROGEN RECEPTOR ALPHA AGONIST TO
THE MALE RAT MEDIAL PREOPTIC AREA MAINTAINS MATING
BEHAVIOR**

by

BINIYAM HABTEAB

Under the Direction of Andrew N. Clancy, PhD

ABSTRACT

Evidence from knockout studies in male mice and from experiments in male rats, in which expression of the estrogen receptor alpha (ER α) gene was inhibited in the medial preoptic area (MPO), suggests that ER α is important in the control of male rat mating behavior. Therefore, in this experiment, we tested the hypothesis that activation of ER α in the MPO is sufficient to maintain mating behavior in castrated male rats receiving subcutaneously (s.c.) dihydrotestosterone (DHT), a non-aromatizable androgen. Accordingly, castrated rats treated with DHT s.c. received MPO implants of either: (i) propyl-pyrazole-triol (PPT) (Stauffer, *et al* 2000; Katzenellenbogen, *et al* 2000), a selective ER α agonist, (ii) E₂ (positive controls) or (iii) cholesterol (negative controls) and sexual behavior was monitored. PPT was as effective as E₂ at maintaining mating behavior suggesting that, in the MPO, ER α is sufficient to mediate responses to E₂ that underlie male rat mating behavior.

INDEX WORDS: Medial preoptic area, Estrogen receptor, Estradiol, Dihydrotestosterone, propyl-pyrazole-triol (PPT), Cholesterol, Sexual behavior

**IMPLANT OF A SELECTIVE ESTROGEN RECEPTOR ALPHA AGONIST TO
THE MALE RAT MEDIAL PREEPTIC AREA MAINTAINS MATING
BEHAVIOR**

by

BINIYAM HABTEAB

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of
Master of Science
in the College of Arts and Sciences
Georgia State University

2007

Copyright by
BINIYAM SEGED HABTEAB
2007

**IMPLANT OF A SELECTIVE ESTROGEN RECEPTOR ALPHA AGONIST TO
THE MALE RAT MEDIAL PREOPTIC AREA MAINTAINS MATING
BEHAVIOR**

by

BINIYAM HABTEAB

Major Professor:
Committee

Andrew Clancy
Delon Barfuss
Matthew Grober

Electronic Version Approved:

Office of Graduate Studies
College of Arts and Sciences
Georgia State University
May 2007

ACKNOWLEDGMENTS

I am extremely grateful to have Dr. Andrew Clancy as my thesis advisor and for opening the door of opportunity. I like also to thank him for his leadership and team spirit, provided support during my training and his patience and enthusiasm shaped my writing and oral presentation skills.

I also express my appreciation to the members of committee Dr. Delon Barfuss, and Dr. Matthew Grober, for their time, effort and for their recommendations that shaped my thesis writing.

I thank Carrie Paisley for training and for her friendship. I would like to thank my laboratory partners and friends Meron Tesfay, Hodan Ahmed, Britani Hall, Binyam Tefera, Faustina Donkor, Aisha Saibo, and Harshasu Barot.

I thank LaTasha Warren-Morrison for lending helping hands when I needed it and for helping out with graduate school procedures. I am also grateful for Dr. Bill Walthall for outstanding advices and guidance for the last two years.

Finally, I would like to thank entire family especially my sister Stegab Habteab, for unshakable support. I would like to thank my parents Habteab Segid and Genet Tekle and all my relatives and my friends in U.S.A. and Eritrea.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	iv
LIST OF FIGURES	vi
LIST OF ABBREVIATIONS AND SYMBOLS	vii
INTRODUCTION	1
MATERIALS AND METHODS	4
Animals	4
Female rats surgical procedures	5
Male behavior, screening, surgery, and testing	5
Histology	7
Statistical analyses	7
RESULTS	8
Behavior	8
Hisstology	11
DISCUSSION	18
LITRATURE CITED	28

LIST OF FIGURES

FIGURE 1: Mount frequency, intromission frequency, ejaculation frequency, and hit rate percentage.	13
FIGURE 2: Mount latency, inter-mount interval, ejaculatory latency, and post ejaculatory interval.	15
FIGURE 3: Cannulae Placement	17
FIGURE 4: PPT binds to ER α but not ER β	21

LIST OF ABBREVIATIONS AND SYMBOLS

AD	Alzheimer's disease
AH	anterior hypothalamus
NOVA	analysis of variance
ApoE2	apolipoprotein E2
ApoE3	apolipoprotein E3
ApoE4	apolipoprotein E4
AR	Androgen receptors
AS-ODN	Antisense oligodeoxynucleotide)
ATD	1,4, 6-androstatriene-3,17-dione
BST	Bed nucleus of the stria terminalis
cAMP	cyclic adenosine mono phosphate
C1	carbon one
C2	carbon two
C3	carbon three
C4	carbon four
C5	carbon five
N1	nitrogen one
DAG	diacylglycerol
DA	dopamine
D ₁	dopamine receptors 1
D ₂	dopamine receptors 2

Df	Degrees of freedom
DHT	Dihydrotestosterone
DHTP	Dihydrotestosterone propionate
DPN	diarylpropionitrile
E ₂	Estradiol
E ₂ .BSA	E ₂ -bovine serum albumin
EB	Estradiol benzoate
EF	Ejaculation frequency
EL	Ejaculation latency
ER	Estrogen receptor
ER α	Estrogen receptor alpha
ER β	Estrogen receptor beta
ER α KO	Estrogen receptor alpha knockout
ER β KO	Estrogen receptor beta knockout
$\alpha\beta$ ERKO	Alpha and beta estrogen receptor knockout
G6PDH	glucose-6-phosphate dehydrogenase
HB-EGF	heparin-binding epidermal growth factor
HR	Hit rate
i.d.	inside diameter
IF	Intromission frequency
IMI	inter-mount interval
LDL	low-density lipoprotein

L-MMA	N-monomethyl-L-arginine
LPM	Liter per minute
M	Mean
mm	Millimeter
MEA	Medial amygdale
MF	Mount frequency
ML	Mount latency
MPO	Medial preoptic area
NOS	nitro oxide synthase
nNOS	neuronal nitro oxide synthase
o.d.	outside diameter
p	Probability associated with the occurrence under the null hypothesis a
PEI	Post ejaculatory intervals
PLC	phospholipase C
PPD	propylpyrazole diol
PPT	Propyl-pyrazole-triol
SEM	Standard error of mean
s.c.	Subcutaneously
t	Computed value of t test
T	Testosterone
TP	Testosterone propionate
<	Less than

= Equal to

**IMPLANTS OF A SELECTIVE ESTROGEN RECEPTOR ALPHA AGONIST TO
THE MALE RAT MEDIAL PREOPTIC AREA MAINTAINS MATING
BEHAVIOR**

INTRODUCTION

The medial preoptic area (MPO) is located in the forebrain in the rostral hypothalamus and plays a key role in the expression of male mating behavior (Simerly, 2002). Male rats with MPO lesion showed highly suppressed levels of mounting and intromission and are completely unable to ejaculate (Liu *et al.*, 1997), suggesting that the MPO is necessary for ejaculation. Similarly, lesions of the male rat medial amygdala (MEA) also compromise mating behavior by reducing mounting and intromission, consistent with the possibility that motivation and sexual arousal are decreased (Kondo, 1992). Both testosterone (T) and its androgenic (DHT) and estrogenic (E₂) metabolites act in the MPO and both types of metabolites are necessary for mating behavior to occur (Morali, 1986; Christensen *et al.*, 1986). Implant of testosterone propionate (TP) in the MPO maintains copulatory behavior in castrated male rats including mounting, intromission, and ejaculation (Morali, 1986), as do MPO implants of estradiol (E₂) (if an androgen source is present) (Michael *et al.*, 1973; Baum *et al.*, 1973; McGinnis *et al.* 1989) and dihydrotestosterone (DHT) (if an estrogen source is present) (McGinnis, 1989; Naftolin *et al.*, 1972; Baum, 2003). Under normal circumstances, T is converted into E₂ by aromatase in the brain (Powers *et al.*, 1987; Naftolin *et al.*, 1972) and aromatase

mRNA is expressed in the MPO (Roselli *et al.*, 2000). Testosterone is also converted in the testis or the brain into DHT by 5 alpha-reductase (Whalen *et al.*, 1985; Martini, 1982; Massa, *et al* 1981). Castrated rats treated with either E₂ alone or DHT alone fail to express the full repertoire of copulatory behavior (Wallis *et al.*, 1975; Per Sodersten, 1973). However, castrated rats displayed normal mating behavior to ejaculation after being treated with a combination of E₂ and DHT and their mating behavior was equal to that of rats treated with T (Michael *et al.*, 1973; Baum *et al.*, 1973). Implants of 1,4,6-androstatriene-3,17-dione (ATD), a steroidal aromatase inhibitor, plus TP in the MPO hinder copulatory behaviors, but implant of ATD with estradiol benzoate (EB) in the MPO promote copulatory behavior (Watson *et al.*, 1989). It is worth mentioning, however, that ATD may compete for receptor binding with steroids (Kaplan *et al.*, 1989). Furthermore, gonadectomized male rats treated s.c. with the combination of T and fadrozole, a non-steroidal aromatase inhibitor that blocks the conversion of testosterone to E₂, failed to show copulatory behavior and exogenous E₂ partially reversed this (Bonsall *et al.*, 1992; Roselli, *et al* 2003; Vagell *et al.*, 1997). Similarly, infusion of fadrozole to the MPO of gonadally intact male rats inhibited mating (Clancy, 1995), whereas gonadally intact male rats treated with fadrozole s.c. together with E₂ implants in the MPO displayed normal copulatory behavior (Clancy, 2000). Collectively, these findings suggest that E₂ acts in the MPO and that this action is necessary to maintain sexual behavior in male rats (Clancy *et al.*, 2000; Clancy *et al.*, 1995). The rat MPO

contains more than one type of estrogen-sensitive neuron; some express estrogen receptor alpha ($ER\alpha$) (Shughrue et al., 1997; Kumar *et al.*, 2006) others express estrogen receptor beta ($ER\beta$) (Krege et al., 1998; Greco, 1998) some express both $ER\alpha$ and $ER\beta$ (Abraham *et al.*, 2004; Nomura *et al.*, 2003), others express an ER in the membrane that is G-protein-coupled (Sinchak *et al.*, 2001). After treatment with TP and dihydrotestosterone propionate (DHTP), castrated male mice with estrogen receptor α knockout ($ER\alpha$ KO) were able to mount and intromit, but were unable to ejaculate, suggesting a role for $ER\alpha$ in control of ejaculation (Ogawa *et al.*, 1998; Scordalakes *et al.*, 2002). In contrast to $ER\alpha$ KO, estrogen receptor β knockout ($ER\beta$ KO) castrated male mice showed relatively normal sexual behavior, similar to that of castrated wild type mice treated with TP and DHTP (Ogawa *et al.*, 1999) suggesting $ER\beta$ is not necessary. However, mice with combined $\alpha\beta$ ERKO failed to mate at all (Ogawa *et al.*, 2000), so both $ER\alpha$ and $ER\beta$ may play a role in the display of mating behavior. Placement of an antisense oligodeoxynucleotide (AS-ODN) complementary to $ER\alpha$ mRNA into the MPO inhibits the expression of $ER\alpha$ and reduces levels of mounting, intromission and ejaculation, unlike control rats that had been infused in the MPO with saline or received AS-ODN in the MEA, which mated normally to ejaculation (Paisley *et al.*, 2006). This suggests that E_2 acts differently in different brain regions, $ER\alpha$ in MPO is necessary for male rat mating behavior, and $ER\alpha$ is not necessary in the MEA (Paisley *et al.*, 2006).

Collectively, the evidence from these studies suggest a major role for $ER\alpha$ in the

control of male rat copulatory behavior and a lesser role for ER β , but additional research is required to determine the exact roles of these receptor subtypes in the control of mating and to determine where in the brain each subtype may play a role. We hypothesize that ER α in the MPO is sufficient to promote copulatory behavior in castrated male rats treated s.c. with DHT. MPO implants of PPT were used rather than systemic administration of PPT because we hoped to test where in the brain PPT might act. Therefore, we compared the effects on ejaculation of implants to the MPO of either E₂ or propyl-pyrazole triol (PPT) (Stauffer *et al.*, 2000), a selective estrogen receptor α agonist, in sexually experienced castrated male rats receiving DHT s.c., which supplied the whole brain with a necessary non-aromatizable androgen source. We predicted that PPT would be as effective as E₂ at maintaining male rat mating behavior, demonstrating the behavioral relevance and sufficiency of ER α activation in the MPO.

MATERIALS AND METHODS

Animals

Thirty Sprague Dawley male rats and fifteen female rats were allowed free access of food and water and kept in cages (22 X 44 X 50 cm) in the Georgia State University vivarium, at a temperature between 20⁰-23⁰ Centigrade and humidity of 43-56%. The rats were maintained on 14:10 hours reverse light: dark cycle (light off at 9:30 AM EST). Male rats lived two per cage until surgery after which they are housed singly and

females were housed two per cage throughout the study. Animal care was in accordance with humane standards (NIH publ. No. 85-23, revised 1985) and all procedures involving animals were authorized by the Georgia State University IACUC.

Female rats surgical procedures

Stimules females were anesthetized with isoflurane gas in a chamber (5% gas and 5 LPM oxygen exchange rate) and transferred to a nosecone (2-3% gas and 3 LPM oxygen). Ovaries were removed through an abdominal incision and rats were implanted in the scapular region s.c with a Silastic capsule (6mm length, 1.981 mm i.d. x 3.175 mm o.d.) containing crystalline E₂ (Clancy *et al.*, 1995). Females were allowed at least five days to recover from surgery before they were paired with male rats to measure sexual behavior in the males. Four hours prior to each weekly behavioral test, females were injected s.c. with 1 mg progesterone in 0.2 ml of sesame oil to induce sexual receptivity.

Male behavior screening, surgical procedures, and testing

Thirty male rats were screened once weekly for three consecutive weeks for the display of sexual behavior prior to being selected in the experiment. Tests for sexual behavior were conducted by blind observers once a week, 30 minutes per test. Sex testing took place under red light illumination during the dark phase of the light: dark cycle. The recorded behaviors included: mount frequency (MF): number of mounts with no penetration, intromission frequency (IF): numbers of intromission with penetration, ejaculation frequency (EF): numbers of ejaculation, mount latency (ML): time from test

start until the first mount or intromission or 1800 seconds if mounts did not occur, ejaculation latency (EL): time from first mount or intromission until the first ejaculation or 1800 seconds if ejaculation did not occur, post ejaculatory interval (PEI): the time between the first ejaculation and the next mount or intromission or 1800 seconds if ejaculation did not occur, hit rate (HR): the percentage of mounts accompanied by intromission and inter-mount interval (IMI): average time between consecutive mounts or intromissions during the first mount bout or 1800 for non-ejaculations. (Clancy, A. N., Zumpe, D., and Michael, R. P. 2000; Liu, Y., Salamone, J. D., and Sachs, B. D. 1997). Thereafter, male rats were assigned into three matched groups based on ejaculation frequency: (i) PPT (n=7), (ii) E₂ (n=8) or (iii) cholesterol (n=7). All three groups were implanted in the MPO with the appropriate drug. Specifically, males were anesthetized with isoflurane gas in a chamber (5% gas and 5 LPM oxygen exchange) and transferred to a nosecone (2-3% gas and 3 LPM oxygen exchange). Anesthetized rats were castrated through abdominal incisions and received s.c. a Silastic capsule containing crystalline DHT (15 mm length, 1.981 mm i.d. and 3.175 mm o.d.) in the scapular region. They were then placed in stereotaxic instrument and implanted bilaterally with 22 gauge guide cannulae aimed at the MPO. (Level skull coordination: anterior posterior = -0.5 mm, medial lateral = ± 0.75 mm, dorsal ventral = - 8.0 mm Swanson et al., 1998). 28 gauge inner cannulae, extended 1 mm below guide cannulae. These were tamped in either E₂, PPT or cholesterol to deliver the drug into the MPO. Thereafter, rats were allowed at

least five days to recover from surgery and postoperative behavioral testing began. Sterile inner cannulae were replaced 48 hours before and 24 hours after each behavior test under brief isoflurane anesthesia. Tips of cannulae were examined microscopically after they were removed from the brain to insure drug was present and all cannulae passed this inspection.

Histology

After completion of behavior testing, cannulae placements were confirmed by histology. Males were euthanized with lethal doses of Nembutal, (sodium pentobarbital 12 ml/kg, i.p, Abbott Laboratories), and transcardially perfused with saline, followed by 300 ml of fixative (4% paraformaldehyde, in 0.1 M phosphate buffer). Brains were then immersed in fixative for at least 24 hours, transferred to 30% sucrose in 0.1 M phosphate buffer for at least two days and cut coronally into 40 μm sections on a freezing microtome. Sections were stained with thionin and locations of the cannulae tips were mapped as black spots onto standard atlas plates.

Statistical analyses

Repeated measures (groups x trials) analysis of variance (ANOVA) was used for statistical comparisons among the groups (Kirk, 1968). Follow-up post-hoc analysis (Tukey honestly significant differences test, $\alpha = 0.05$) was used to identify significant group differences. Paired t-tests were used to compare within group changes in each group between the third pre-operative behavior test (terminal pre-operative performance)

and the fourth post-operative behavior test (terminal post-operative performance). Two tailed probabilities are reported in all cases.

RESULTS

Behavior

Both estradiol and PPT implants to the MPO maintained the mating behavior of sexually experienced, castrated male rats receiving DHT s.c. but cholesterol MPO implants were ineffective. Thus, the two groups receiving either estradiol or PPT MPO implants mated vigorously and were statistically indistinguishable from each other on all measures of copulatory behavior during both the preoperative and postoperative periods; moreover, sexual behavior did not change significantly in either of these two groups in the periods before and after surgery. In contrast, sexually experienced, castrated, DHT-treated males that received MPO cholesterol implants virtually ceased mating during the postoperative period and was significantly lower than that of either of the other two groups on all measures of copulatory behavior, although, during the preoperative period, they had copulated robustly and did not differ significantly on any behavioral measure from the rats in the other two groups. Moreover, all measures of sexual behavior declined significantly in the cholesterol MPO group during the period after surgery relative to preoperative levels.

During the postoperative period (FIGURE 1), the groups differed significantly from each other on mount frequency ($F_{2,19} = 10.23$, $p < 0.001$), intromission frequency ($F_{2,19} = 10.80$, $p < 0.001$), ejaculation frequency ($F_{2,19} = 10.98$, $p < 0.001$) and hit rate

($F_{2,19} = 9.31$, $p < 0.002$). Moreover (FIGURE 2), during the postoperative period, the groups also differed significantly from each other on mount latency ($F_{2,19} = 13.80$, $p < 0.0001$), ejaculation latency ($F_{2,19} = 6.66$, $p < 0.007$), inter-mount interval ($F_{2,19} = 6.86$, $p < 0.006$) and the post-ejaculatory interval ($F_{2,19} = 8.12$, $p < 0.003$). No other statistically significant main effects or interactions were observed during the postoperative period. Follow-up post-hoc analysis of behavior during the postoperative period revealed no significant differences on any test day between the estradiol and PPT groups on any of the eight behavioral measures, however, mount frequency in the cholesterol group was significantly lower than that in each of the other two groups on the second ($p < 0.05$) and third ($p < 0.05$) days; on intromission frequency, the cholesterol group was significantly lower ($p < 0.05$) than the estradiol group on all four trials and lower than the PPT group on the second and fourth trials ($p < 0.05$, respectively); and on hit rate, the cholesterol group was significantly lower ($p < 0.05$) than the PPT group on the fourth day (FIGURE 1). Similarly (FIGURE 2), during postoperative trials, the cholesterol group exhibited significantly longer mount latencies than the estradiol group on the second, third and fourth days and PPT group on the second and fourth days ($p < 0.05$, respectively); on ejaculation latency, the cholesterol group was significantly ($p < 0.05$) higher than the estradiol group on the second day; and with respect to the inter-mount interval and the post-ejaculatory interval on the second postoperative trial, the cholesterol group showed significantly longer latencies than both the estradiol and PPT groups ($p < 0.05$, respectively).

There were no significant group differences on any of the eight measures of copulatory behavior during the preoperative trials, nor were there any significant interactions. Significant behavioral differences emerged, however, as the days of testing progressed during the preoperative period. Specifically, mount frequency scores ($F_{2,38} = 24.76$, $p > 0.0001$) and intromission frequency scores ($F_{2,38} = 22.93$, $p > 0.0001$) improved significantly as preoperative testing advanced (FIGURE 1), whereas mount latency scores decreased significantly ($F_{2,38} = 12.90$, $p > 0.0001$) during the preoperative period.

Changes in sexual behavior within each group were examined by comparing the performances on the last preoperative testing day and the last postoperative testing day. There were no significant changes on any of the eight indices of copulatory behavior in the groups that received MPO implants of either estradiol or PPT, however, on all eight measures of mating, sexual behavior deteriorated significantly during the postoperative period in the group implanted with cholesterol to the MPO. Specifically, in the cholesterol group, mount frequency ($t = 8.15$, $df = 6$, $p < 0.0002$), intromission frequency ($t = 6.05$, $df = 6$, $p < 0.0009$), ejaculation frequency ($t = 4.80$, $df = 6$, $p < 0.003$) and hit rate ($t = 2.84$, $df = 6$, $p < 0.03$) were significantly depressed on the last postoperative test compared with the last preoperative day (FIGURE 1), whereas mount latency ($t = 3.99$, $df = 6$, $p < 0.007$), ejaculation latency ($t = 2.88$, $df = 6$, $p < 0.03$), inter-mount interval ($t = 3.94$, $df = 6$, $p < 0.007$) and the post-ejaculatory interval ($t = 4.73$, $df = 6$, $p < 0.003$) were significantly lengthened on the last postoperative test relative to the last preoperative day (FIGURE 2).

Histology

Locations of the cannulae tips were mapped as black spots onto standard atlas plates; all cannulae tips were located in the MPO or anterior hypothalamus (FIGURE 3).

FIGURE 1: Mount frequency, intromission frequency, ejaculation frequency, and hit rate percentage.

Mean \pm standard error of the mean (SEM) mount frequency, intromission frequency, ejaculation frequency, and hit rate in weekly, 30 min sex tests. During the post operative but not preoperative periods the groups differed significantly on each of these behavioral measure (See text for F values).

*: Between-group comparison: cholesterol differs significantly from E₂, $p < 0.05$.

+: Between-group comparison: cholesterol differs from PPT, $p < 0.05$.

X: Cholesterol within group comparison, $p < 0.05$.

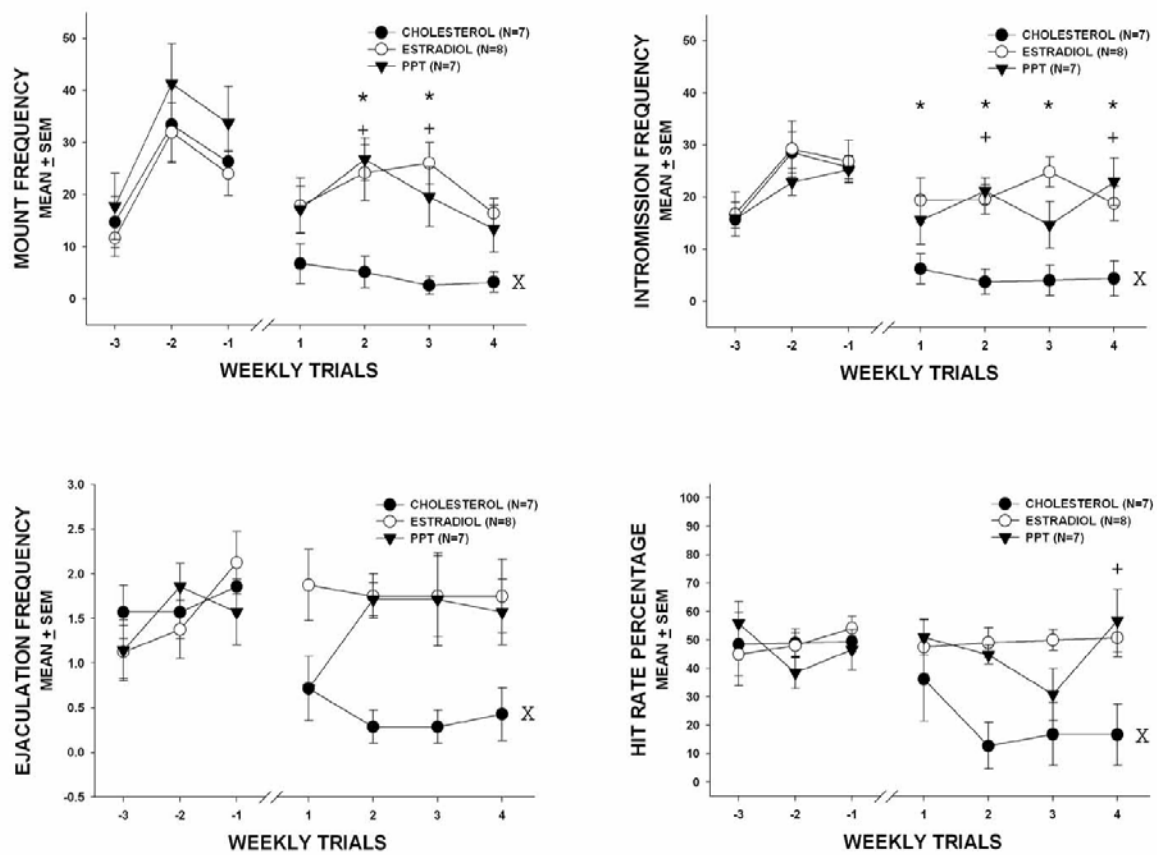


FIGURE 1

FIGURE 2: Mount latency, inter-mount interval, ejaculatory latency, and post ejaculatory interval.

In seconds, mean \pm SEM mount latency, inter-mount interval, ejaculatory latency, and post ejaculatory interval in weekly, 30 min sex tests. During the post operative but not preoperative periods the groups were differed significantly on each of these behavioral measure (See text for F values).

*: Between-group comparison: cholesterol differs significantly from E₂, $p < 0.05$.

+: Between-group comparison: cholesterol differs from PPT, $p < 0.05$.

X: Cholesterol Within group comparison, $p < 0.05$.

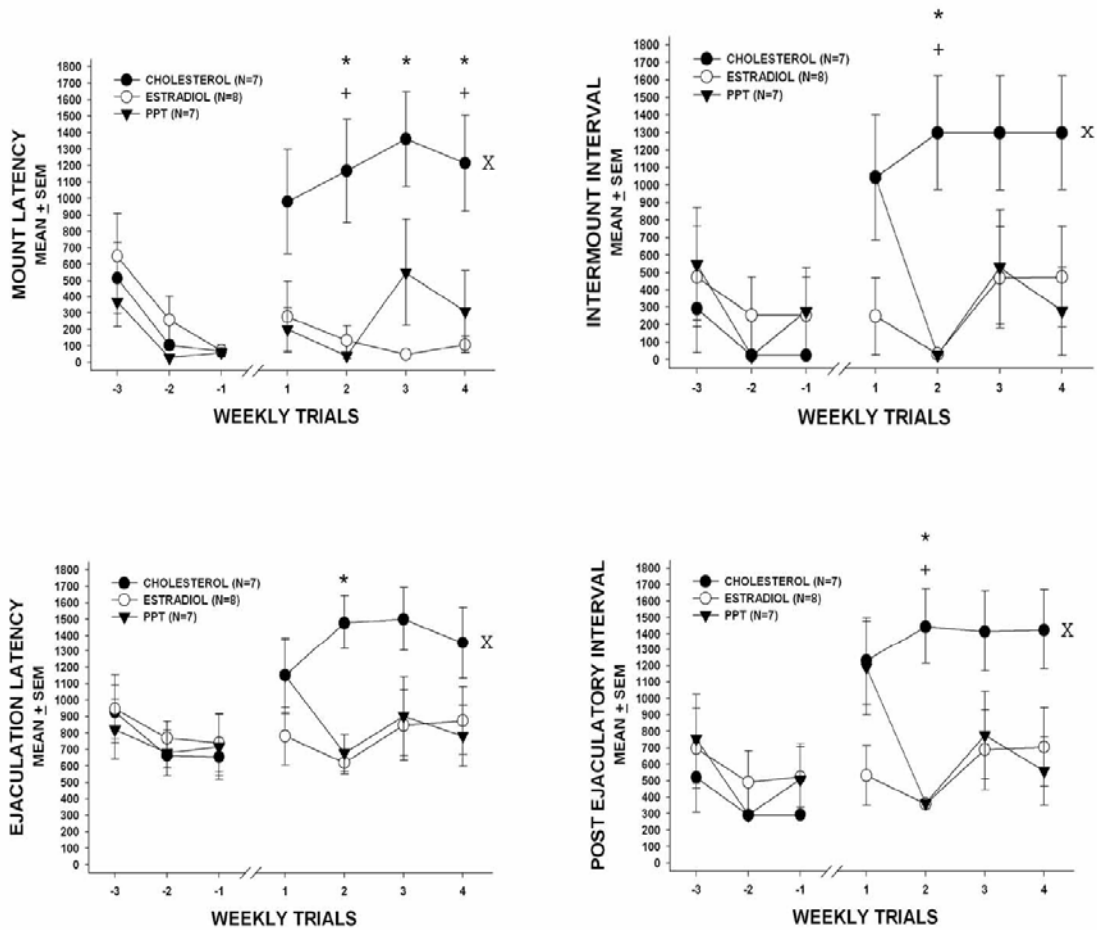


FIGURE 2

FIGURE 3: Cannulae Placement.

The cannulae tips were located in the MPO or anterior hypothalamus (AH). The distribution of cannulae tips in the MPO was mapped on a standard series of coronal atlas plates through the MPO and anterior hypothalamus. Dots represent locations of cannulae tips from each animal.

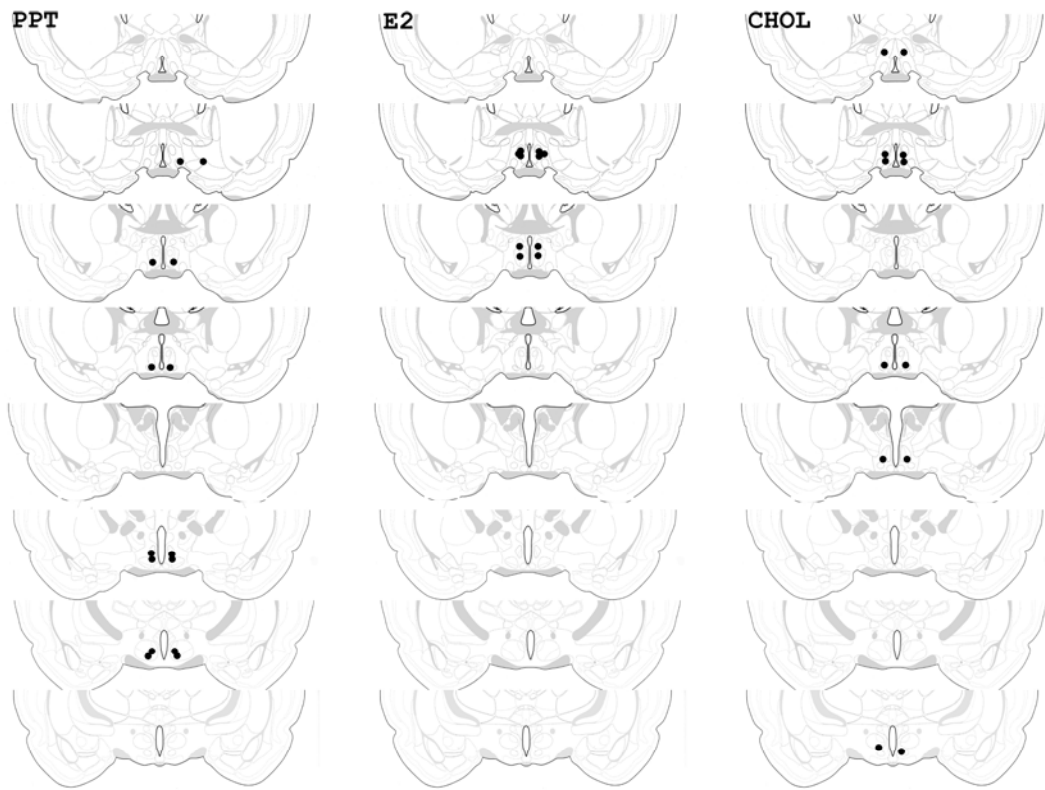


FIGURE 3

DISCUSSION

In this study, we compared the copulatory behavior of sexually experienced, castrated rats treated with DHT s.c that received MPO implants of either PPT, an estrogen receptor α agonist, E_2 or cholesterol. PPT treated rats displayed comparable levels of mating behavior to those treated with E_2 , whereas mating virtually ceased in cholesterol treated animals as shown in other studies (Rosenblatt *et al.*, 1998). These findings support the hypothesis that ER α in the MPO is sufficient to promote mating behavior in castrated male rats treated s.c. with DHT. PPT is thought to activate ER α only whereas E_2 activates both ER α and ER β yet rats treated with PPT mounted, intromited and ejaculated at rates that were statistically indistinguishable from those treated with E_2 . In the cholesterol group, on the other hand, presumably neither ER α nor ER β were activated and these rats virtually ceased mating.

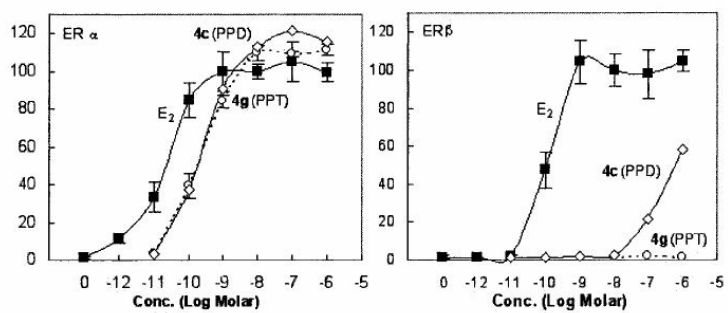
A critical region of the brain for male copulation control is the MPO, as much prior research has shown (Clancy *et al.*, 2000; Clancy, et al., 1995; Bonsall et al., 1992; Morali, 1986). Steroid sensitive neurons in the MPO are activated by mating (Greco *et al.*, 1998), including those expressing androgen receptors (Greco *et al.*, 1999), ER α and ER β (Shughrue et al., 1997; Kumar *et al.*, 2006; Krege et al., 1998; Greco, 1998) and there is much evidence suggesting that ER α is the behaviorally relevant ER (Ogawa *et al.*, 1998; Scordalakes *et al.*, 2002; Paisley *et al.*, 2006). The present results demonstrate that activation of ER α in the MPO is sufficient to promote copulatory behavior in castrated DHT treated male rats. Specifically implants of the MPO with PPT, a selective

ER α agonist, supported mating equally as well as E₂, which stimulates both estrogen receptor α and β .

In human cell culture, PPT binds ER α with the same affinity as E₂ binds ER α and has zero cross reactivity with ER β (Stauffer *et al.*, 2000) (FIGURE-4).

FIGURE 4: PPT binds to ER α but not ER β .

“Transcription activation by ER α (left) and ER β (right) in response to pyrazole 4c (PPD) and 4g (PPT). Human endometrial cancer (HEC-1) cells were transfected with expression vectors for ER α or ER β and an (ERE) 3-pS-CAT reporter gene and were treated with the indicated concentrations of ligand for 24 h. CAT activity was normalized for β -galactosidase activity from an internal control plasmid. Values are expressed as a percent of the ER α or ER β response with 2 nM E₂, which is set at 100%” (Stauffer *et al.*, 2000).



Stauffer *et al.*, 2000

FIGURE 4

Ligands such as PPT or estradiol bind selectively to receptors depending on the ligand binding pocket, cofactors, estrogen response elements and their promoters and other factors (Wright *et al.*, 2006). For example, ER α has in its ligand binding pocket Met 421 and ER β has Ile 373; the ER α ligand binding pocket is occupied by Leu384, on the other hand, ER β has Met 336 (Wright *et al.*, 2006; Katzenellenbogen *et al.*, 2000). PPT is formed from pyrazole attached at C3 and C5 with phenol, N1 phenyl and C4 with propyl group (Wright *et al.*, 2006). The hydroxyl portion of the phenol group at C3 and C5 have no charge and can react with the hydrophilic side chain of other compounds in order to form hydrogen bonds (Stauffer *et al.*, 2000). Both the nitrogen groups at C1 and C2 also have no charge and they can form a hydrogen bond with hydrophilic side chain of the receptor proteins or water (Stauffer *et al.*, 2000).

In vitro experiments demonstrate that ER α , but not ER β , bind to PPT. Relative binding affinity is measured after replacing the R (alkyl group) with different alkyl groups and the X (halogen group) is replaced with hydrogen or hydroxy groups (Stauffer *et al.*, 2000). These competitive radiometric binding assays were measured in human ER α and ER β (Stauffer *et al.*, 2000). The assay showed that PPT has greater affinity toward ER α by 410 fold than to ER β (Stauffer *et al.*, 2000). The ability of PPT to activate transcription of ER α or ER β genes were demonstrated in human endometrial cancer cells (Stauffer *et al.*, 2000). The potency selectivity between ER α /ER β to PPT indicates that PPT binds to ER α with greater potency than ER β (Stauffer *et al.*, 2000).

PPT has been used in various experiments as an ER α agonist. For instance, PPT activates ER α , whereas diarylpropionitrile (DPN) activates ER β , in hippocampus *in vitro*

and *in vivo* (Wang *et al.*, 2006). Activation of ER α via PPT promotes the formation of apolipoprotein E4 (ApoE4) which is associated with Alzheimer's disease (AD), triggering an increase in low-density lipoprotein (LDL), and neurodegenerative disease (Harris *et al.*, 2003). On the other hand, the ER β agonist, DPN, inhibits ApoE4 and activates apolipoprotein E2 (ApoE2) and apolipoprotein E3 (ApoE3), which is linked to a reduced risk of AD (Wang *et al.*, 2006). Similar observations were also seen in immature mice that had been injected with PPT; PPT increased uterus weight (Frasor *et al.*, 2003). Collectively these studies demonstrate that PPT activates at least three different genes that include complement component 3 (C3), lactoferrin, and glucose-6-phosphate dehydrogenase (G6PDH) in the uterus (Frasor *et al.*, 2003).

Our new finding extends previous reports showing that blockade of ER α expression in the MPO impairs mating behavior (Paisley *et al.*, 2006). Specifically infusion of the MPO with an antisense oligodeoxynucleotide sequence complementary to ER α mRNA reduced expression of ER α and sexual behavior in male rats (Paisley *et al.*, 2006), suggesting a role for ER α in control of mating behavior. Similarly, male ER α knockout mice also show reduced mating behavior (Ogawa *et al.*, 1998; Scordalakes *et al.*, 2002). The present findings complement these earlier reports by demonstrating that a selective ER α agonist drug maintains behavior equally as well as E₂, which suggests the behaviorally relevant ER in the male rat MPO is ER α with respect to mating behavior.

Each behavior we observed, MF, IF, EF, ML, EL, PEI, HR and IMI, is maintained by E₂ activation of ER in the MPO and specifically by ER α , since PPT MPO implants

were as effective as E₂ MPO implants at promoting mating in gonadomatized male rats treated with exogenous DHT, but cholesterol MPO implants were ineffective.

It is pertinent that T is converted into E₂ through aromatization (Naftolin *et al.*, 1972; Powers *et al.*, 1987), and DHT is formed from T through the action of 5- α reductase (Martini, 1982; Massa, *et al.*, 1981; Whalen *et al.*, 1985). Exogenous T in physiological concentrations is sufficient to promote copulatory behavior in castrated male rats, and combined treatment with E₂ and DHT, each in physiological concentration, is as effective as T in promoting sexual behavior (Michael *et al.*, 1973; Baum *et al.*, 1973; McGinnis *et al.* 1989; McGinnis, 1989; Naftolin *et al.*, 1972; Baum, 2003). In contrast, sexual behavior is poorly maintained by either E₂ alone or DHT alone except if substantially in higher doses in the pharmacological range are administered (McGinnis, 1989). It is noteworthy that T must be converted into E₂ and DHT to maintain sexual behavior in castrated rats and if this conversion of T to E₂ is blocked, then sexual behavior is depressed (Naftolin *et al.*, 1972; Baum, 2003; Bonsall *et al.*, 1992). E₂ acts in the brain by binding to estrogen receptors whereas T and DHT bind to androgen receptors. Both estrogen receptors and androgen receptors are found in different regions of the brain, including the MPO, supraoptic nucleus, ventral zone of medial parvicellular part of the paraventricular nucleus of hypothalamus and lateral cerebellar region (Simerly *et al.*, 1990). Since, both ER and AR reside within the MPO (Handa *et al.*, 1996), both ER and AR containing neurons have the potential to mediate male rat mating.

There are at least two ways that E₂ may affect its targets, either by activating the genome or via a non-genomic pathway; many questions remain concerning how estrogen

acts in the MPO. One neuronally mediated estrogen dependent response that has received considerable attention is the estrogen activation, via ER α , of MPO dopamine circuits (Scordalakes *et al.*, 2002; Putnam *et al.*, 2005). It has been proposed that E₂ activation of ER α enhances the formation of neuronal nitro oxide synthase (nNOS) (Scordalakes *et al.*, 2002) which converts L-arginine into citrulline and nitric oxide (NO) (Palmer *et al.*, 1988). NO, in turn, triggers the release of dopamine (DA) (Scordalakes *et al.*, 2002;) in the MPO. This increase in dopamine in the MPO elicits erection and ejaculation (Hull *et al.*, 1992; Hull *et al.*, 1989; Dominguez *et al.*, 2004; Hull *et al.*, 1999; Putnam *et al.*, 2005). There is also a negative feed back mechanism whereby elevated levels of NO inhibit aromatase, which limits further production of E₂ from T (Snyder *et al.*, 1996). Castrated animals tend to have decreased nNOS but exogenous administration of T or E₂ reverses this effect of castration and nNOS subsequently increases (Du, *et al.*, 1999; Putnam *et al.*, 2005). Furthermore, castration also decreases the release of DA from the MPO and this is due to lack of NO in the MPO (Du, *et al.*, 1998). Immunocytochemical studies have demonstrated the coexpression of AR, ER α and nNOS in the MPO (Sota *et al.*, 2005). Implantation of the nitro oxide synthase (NOS) inhibitor, N-monomethyl-L-arginine (L-NMMA), blocked the expression of NO in the MPO and reduced the mount rate. Conversely, administration of L-arginine, a NO precursor, in the MPO promoted an increase of mounting in male rats (Sota *et al.*, 1998), whereas blocking the synthesis of NOS in an intact rat inhibits the formation of DA and lowered mating behavior (Hull *et al.*, 1997).

Castrated male rat failed to achieve erection following electrical stimulation of MPO, but implant of T in the MPO reinstated erection (Suzuki *et al.*, 2007; Giuliano, 1997). This suggests that MPO plays a role in an erection in response to T (Suzuki *et al.*, 2007). Stimulation of dopamine receptor 1 (D₁) in the MPO enhanced erection by activating parasympathetic pathways, and dopamine receptor 2 (D₂) in the MPO operating through a sympathetic pathway to elicit ejaculation (Hull *et al.*, 1992; Hull *et al.*, 1989; Dominguez *et al.*, 2004).

In the conclusion, there is much evidence suggesting that ER α is the behaviorally relevant ER for mating. We demonstrated that activation of ER α in the MPO is sufficient to promote copulatory behavior in castrated DHT treated male rats. We implanted PPT, a selective ER α agonist, in MPO which maintained mating behavior equally as well as E₂, (which stimulates both ER α and ER β). Sexual behavior data gathered from this PPT study suggests that ER α is the ER in the MPO that is sufficient for display of mating behavior. Further investigation is required to identify whether MPO ER acts as a membrane receptor or whether it acts as transcription factor in promoting sexual behaviors. More studies will be required to determine other sites in the brain where PPT may act and to test if its effects in the MPO are specific by incorporating anatomical controls and by examining the MPO more closely to see if PPT has selective effects in different sub regions of the MPO. However, recent finding in our laboratory indicated that castrated, DHT treated male rats implanted with E₂ conjugated to bovine serum albumin (BSA) in the MPO were able to mate normally (Huddleston *et al.*, 2006, Personal communication). This raises the possibility that ER α associated with the plasma

membrane of MPO neurons may mediate the responses to E₂ that underlie male rat copulatory behavior.

LITERATURE CITED

Abraham, I. M., Todman, M. G., Korach, K. S., and Herbison, A. E. (2004). Critical *in vivo* roles for classical estrogen receptors in rapid estrogen actions on intracellular signaling in mouse brain. *Endocrinology*. **145**, 3055-3061.

Baum, J. M., and Vreeburg T. M. (1973). Copulation in castrated male rats following combined treatment with estuarial and dihydrotestosterone. *Science*. **182**, 283-285

Baum, M. J. (2003). Activational and organizational effects of estradiol on male behavioral neuroendocrine function. *Scandinavian Journal of Psychology*. **44**, 213-220.

Bonsall, R. W., Clancy, A. N., and Michael, R. P. (1992). Effects of the nonsteroidal aromatase inhibitor, Fedrozole, on sexual behavior in male rats. *Hormone Behaviors*. **26**, 240-254.

Christensen, L. W. and Clemens, L. G. (1975). Blockade of testosterone-induced mounting behavior in the male rat with intracranial application of the aromatization inhibitor, andros-1,4,6-triene-3,17-dione. *Endocrinology* **97**, 1545-1551.

Clancy, A. N., Zumpe, D., and Michael R. P. (1995). Intracerebral infusion of an aromatase inhibitor, sexual behavior and brain estrogen receptor-like immunoreactivity in intact male rats. (1995). *Neuroendocrinology*. **61**, 98-111.

Clancy, A. N (1998). Androgen receptors and estrogen receptors are colocalized in male rat hypothalamic and limbic neurons that express Fos immunoreactivity induced by mating. *Neuro-endocrinology*. **67**, 18-28.

Clancy, A. N., Zumpe, D., and Michael, R. P. (2000). Estrogen in the medial preoptic area of male rats facilitates copulatory behavior. *Hormones and Behavior*. **38**, 86-93.

Coolen, L. M., and Wood, R. I. (1998). Testosterone stimulation of the medial preoptic area and medial amygdala in the control of male hamster sexual behavior: redundancy without amplification. *Behavioral Brain Research*. **98**, 143-153.

Dominguez, J. M., Muschamp, J. W., Schmich, J. M., and Hull, E. M. (2004). Nitric oxide mediates glutamate-evoked dopamine release in the medial preoptic area. *Neuroscience*. **125**, 203-210.

Du, J., and Hull, E. M. (1999). Effects of testosterone on neuronal nitric oxide synthase and tyrosine hydroxylase. *Brain Research*. **836**, 90-98.

Du, j., Lorrian, D. S., Hull, E. M. (1998). Castration decreases extracellular, but increases intracellular, dopamine in medial preoptic area of male rats. *Brain Research*. **782**, 11-17.

Fernandez-Fewell, G. D., and Meredith, M. (1994). C-fos Expression in vomeronasal pathways of mated or pheromone-stimulated male golden hamsters: contributions from vomeronasal sensory input and expression related to mating performance. *The Journal of Neuroscience*. **14**, 3643-3654.

Frasor, J., Barnett, D. H., Danes, J. M., Hess, R., Parlow, A. F., and Katzenellenbogen, B. S. (2003). Response-specific and ligand dose-dependent modulation of estrogen receptor (ER) α activity by ER β in the uterus. *Endocrinology*. **144**, 3159-3166.

Giuliano, F., Bernabe, J., Brown, K., Droupy, S., Benoit, G., and Rampin, O. (1997) Erectile response to hypothalamic stimulation in rats: role of peripheral nerves. *American Journal of Physiology*. **273**, R1990-R1997.

Greco, B., Edwards, D. A., Mechael, R. P., Zumpe, D., Clancy, A. N. (1999).

Colocalization of androgen receptors and mating-induced FOS immunoreactivity in neurons that project to the central tegmental field in male rats. *Journal of comparative neurology*. **31**, 220-236.

Greco, B., Edwards, D. A., Mechael, R. P., Clancy, A. N. (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.

Handa, R. J., Kerr, J. E., DonCarlos, L. L., McGivern, R. F., and Hejna, G. (1996).

Hormonal regulation of androgen receptor messenger RNA in the medial preoptic area of the male rat. *Brain Research Molecular Brain Research*. **39**, 57-67.

Harris, F. M., Brecht, W. J., Xu, Q., Tesseur, I., Kekonius, L., Wyss-Cory, T.,

Fish, J. D., Masliah, E., Hopkins, P. C., Scarce-Levie, K., Weisgraber, K. H.,

Mucke, L., Mahley, R. W., and Huang, Y. (2003). Carboxyl-terminal-truncated

apolipoprotein E4 causes Alzheimer's disease-like neurodegeneration and

behavioral deficits in transgenic mice. *Medical Sciences*. **100**, 10966-10971.

Hisamoto, K., Ohmichi, M., Kanda, Y., Adachi, K., Nishio, Y., Hayakawa, J., Mabuchi, S., Takahashi, K., Tasaka, K., Miyamoto, Y., Taniguchi, N., and Murata, Y. (2001) Induction of endothelial nitric-oxide synthase phosphorylation by the Raloxifene analog LY 117018 is differentially mediated by Akt and extracellular signal-regulated protein kinase in vascular endothelial cells. *The Journal of Chemistry*. **276**, 47642-47649.

Huddleston, G. G., Paisley, J. C., and Clancy, A. N. (2006). Effects of estrogen in the male rat medial amygdale: infusion of an aromatase inhibitor lowers mating and bovine serum albumin-conjugated estradiol implants do not promote mating. *Neuroendocrinology*. **83**, 106-116.

Hull, E. M., Du, J., Lorrain, D. S., and Matuszewich, L. (1997). Testosterone, preoptic dopamine, and copulation in male rats. **44**, 327-333.

Hull, E. M., Eaton, R. C., Markowski, V. P., Moses, J., Lumley, , L. A., and Loucks, J. A. (1992). Opposite influence of medial preoptic D₁ and D₂ receptors on genital reflexes: implications for copulation. *Life Science*. **51**, 1705-1713.

Hull, E. M., Warner, R. K., Bazzett, T. J., Eaton, R. C., Thompson, J. T., and Scaletta, L. L. (1989). D₂/D₁ ratio in the medial preoptic area affects copulation of male rats. *Journal of Pharmacology Experimental Therapeutics*. **251**, 422-427.

Kaplan, M. E., and McGinnis M. Y. (1989). Effects of ATD on male sexual behavior and androgen receptor binding: a reexamination of the aromatization hypothesis. *Hormones and Behavior*. **23**, 10-26.

Katzenellenbogen, B. S., Choi, I., Mourroux, R. D., Ediger, T. R., Martini, P. G., Montano, M., sun, J., Weis, K., and Katzenellenbogen, J. A. (2000). Molecular mechanisms of estrogen action: selective ligands and receptor pharmacology. *The journal of Steroid Biochemistry and Molecular Biology*. **74**, 279-285.

Krege, J. H., Hodgin, J. B., Couse, J. F., Enmark, E., Warner, M., Mahler, J. F., Sar, M., Korach, K. S., Gustafsson, J., Smithies, O. (1998). Generation and reproductive phenotypes of mice lacking estrogen receptor β . **95**, 15677-15682.

Kumar, V. M., Vetrivelan, R., Mallick, H. N. (2006). Alpha-1 adrenergic receptors in the medial preoptic area are involved in the induction of sleep. *Neurochemical research*. **31**, 1095-1102.

Liu, Y., Salamone, J. D., and Sachs, B. D. (1997). Lesions in the medial preoptic area and bed nucleus of stria terminalis: differential effects on copulatory behavior and noncontact erection in male rats. *The Journal of Neuroscience*. **17**, 5245-5253.

Martini, L. (1982). The 5-alpha reduction of testosterone in the neuroendocrine structures: biochemical and physiological implications. *Endocrine review*. **3**, 1-25.

Massa, R., and Sharp, P. J. (1981). Conversion of testosterone to 5 beta-reduced metabolites in the neuroendocrine tissues of the maturing cockerel. *Journal of Endocrinology*. **88**, 263-269.

McGinnis, M. Y., and Dreifuss, R. M. (1989). Evidence for a role of testosterone-androgen receptor interactions in mediating masculine sexual behavior in male rats. *Endocrinology*. **124**, 618-626.

Michael B. J., and Vreeburg T. M. (1973). Copulation in Castrated Male Rats following Combined Treatment with Estradiol and Dihydrotestosterone. *Science*. **183**, 283-285.

Michael V. E., and Marilyn M. Y. (1997). The Role of Aromatization in the Restoration of Male Rat Reproductive Behavior. *Journal of Neuroendocrinology*. **9**, 415-421.

Morali, G. Hernandez, G. and Beyer, C. (1986). Restoration of the copulatory pelvic thrusting pattern in castrated male rats by the intracerebral implantation of androgen. *Physiology & Behavior*. **36**,495-499.

Naftolin, F., Ryan, K. J., and Petro, Z. (1972). Aromatization of androstenedione by the anterior hypothalamus of adult male and female rats. *Endocrinology*. **90**, 295-298.

Nomura, M., Korach, K. S., Pfaff, D. W., and Ogawa, S. (2003). Estrogen receptor beta (ERbeta) protein levels in neurons depend on estrogen receptor alpha (ERalpha) gene expression and on its ligand in a brain region-specific manner. *Brain Research, Molecular Brain Research*. **110**, 7-14

Ogawa, S., Chan, J., Chester, A. E., Gustafsson, J., Korach, K. S., and Donald, P. W. (1999). Survival of reproductive behaviors in estrogen receptor β gene-deficient (β ERKO) male and female mice. *Neurobiology*. **22**, 12887-12892.

Ogawa, S., Chester, A. E., Hewitt, S. C., Walker, V. R., Gustafsson, J., Smithies, O., Korach, K. S., and Pfaff, D. W. (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**, 14737-14741.

Ogawa, S., Washburn, T. F., Tylor, J., Lubahn, D. B., Korach, K. S., and Pfaff, D. W. (1998). Modifications of testosterone-dependent behaviors by estrogen receptor- α gene disruption in male mice. *Endocrinology*. **139**, 5058-5069.

Paisley, P. C., Huddleston, G. G., Carruth, L. L., Grober, M. S. Petrulis, A Clancy, A. N. (2006). Inhibition of estrogen receptor synthesis in the medial preoptic area, but not the medial amygdale, reduces male rat copulatory behavior. *Journal of Neuroscience*. inpreparation, 2006.

Palmer, R. M., Ashton, D. S., and Moncada, S. (1988). Vascular endothelial cells synthesize nitric oxide from L-arginine. *333*, 664-6.

Per sodersten,. (1973). Estrogen-activated sexual Behavior in Male Rats. *Hormones and Behavior*. **4**, 247-256.

Putnam, S. K., Sato S., and Hull, E. M. (2003). Effects of testosterone metabolites on copulation and medial preoptic dopamine release in castrated male rats. **44**, 419-426.

Putnam, S. K., sato, S., Riolo, J. V., and Hull E. M. (2005). Effects of testosterone metabolites on copulation, medial preoptic dopamine, and NOS-immunoreactivity in castrated male rats. *Hormones and Behavior*. **47**, 513-522.

Roselli, C. E., Horton, L. E., and Resko, J. A. (1985). Distribution and regulation of aromatase activity in the rat hypothalamus and limbic system. *Endocrinology*. **117**. 2471-2477.

Rosenblatt, J. S., and Ceus, K. (1998). Estrogen implants in the medial preoptic area stimulate maternal behavior in male rats. *Hormones and Behavior*. **33**, 23-30

Roselli, C. E., Cross, E., Poonyagariyagorn, H. K., and Stadelman, H. L. (2003). Role of aromatization in anticipatory and consummatory aspects of sexual behavior in male rats. *Hormones and Behavior*. **44**, 146-151.

Roselli, C. E., Stormshak, F., and Resko, J. A. (2000). Distribution of aromatase mRNA in the rat hypothalamus: an in situ hybridization study. *Journal of Neuroendocrinology*. **12**, 656-664

Sato, S., Braham, C. S., Putnam, S. K., and Hull, E. M. (2005). Neuronal nitric oxide synthase and gonadal steroid interaction in the MPOA of male rats: Co-localization and testosterone-induced restoration of copulation and nNOS-immunoreactivity. *Brain Research*. **1043**, 205-213.

Sinchak, K., and Micevych, P. E. (2001). Progesterone blockade of estrogen activation of μ -opioid receptors regulates reproductive behavior. *The Journal of Neuroscience*. **21**, 5723-5729.

Sota, Y., Horita, H., Kurohata, T., Adachi, H., and Tsukamoto, T. (1998). Effect of the nitric oxide level in the medial preoptic area on male copulatory behavior in rats. *American Journal of Regulatory, Integrative and Comparative Physiology.* **274**, R243-247.

Scordalakes, E. M., Imwalle, D. B., and Rissman, E. F. (2002). Oestrogen's masculine side: mediation of mating in male mice. *Reproduction.* **124**, 331-338.

Shughrue, P.J., Lubahn, D. B., Vilar, A. N., Korach, K. S., Merchenthaler, I. (1997). Responses in the brain of estrogen receptor α -disrupted mice. *Neurobiology.* **94**, 11008-11012.

Simerly, R. B. (2002). Wired for reproduction: Organization and development of sexually dimorphic circuits in the mammalian forebrain. *Annual Review neuroscience.* **25**, 507-536.

Simerly, R. B., Chang, C., Muramatsu, M., and Swanson, L. W. (1990). Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. *The Journal of Comparative Neurology.* **294**, 76-95.

Snyder, G. D., Holmes, R. W., Bates, J. N., and Van Voorhis, B. J. (1996). Nitric oxide inhibits aromatase activity: mechanisms of action. *The Journal of Steroid Biochemistry and Molecular Biology*. **59**, 63-69.

Sodersten, P. (1973). Estrogen-activated sexual behavior in male rats. *Hormones and Behavior*. **4**, 247-256. Swanson LW: *Brain maps: structure of the brain*. Ed 2, revised. Amsterdam: Elsevier, 1998.

Stauffer, S R., Coletta, C. J., Tedesco, R., Nishiguchi, G., Carlson, K., Sun, J., Katzenellenbogen, B. S., and Katzenellenbogen, J. A. (2000). Pyrazole Ligand: structure-affinity/activity relationship and estrogen receptor- α -selective agonists. *J. Med. Chem.* **43**, 4934-4947.

Suzuki, N., Sota, Y., Hisasue, S., Kato, R., Suzuki, K., and Tsukamoto, T (2007). Effect of testosterone on intracavernous pressure elicited with electrical stimulation of the medial preoptic area and cavernous nerve in male rats. *Journal of Andrology*. **28**, 218-222.

Wallis C. J., Luttge W. G. (1975). Maintenance of male sexual behavior by combined treatment with oestrogen and dihydrotestosterone in CD-1 mice. *Journal of Endocrinology*. **66**, 257-262.

Wang, J. M., Irwin, R. W., and Brinton, R. D. (2006). Activation of estrogen receptor α increases and estrogen receptor β decreases and apolipoprotein E expression in hippocampus in vitro and in vivo. *Biological Sciences/Neuroscience*. **103**, 16983-16988.

Watson, J. T., and Adkins-Regan, E.(1989). Testosterone implanted in the preoptic area of male Japanese quail must be aromatized to activate copulation. *Hormone Behavior*. **23**, 432-447.

Whalen, R. E., Yahr, P. I., & Luttge, W. G. (1985) The role of metabolism in hormonal control of sexual behavior. In N. Adler, D. Pfaff, & R. W. Goy (Eds.), *Handbook of behavioral neurobiology* (Vol. 7, pp. 609-663). New York: Plenum Press.

Wood, R. I. (1996) Estradiol, but not dihydrotestosterone, in the medial amygdale facilitates male hamster sex behavior. *Physiology and Behavior*. **59**, 833-841.

Wood, R. I., and Newman, S. W. (1995).The Medial Amygdaloid Nucleus and Medial Preoptic Area Mediate Steroidal Control of Sexual Behavior in the Male Syrian Hamster. *Hormones and behavior*. **29**, 338-353.

Wright, K. D., Cavailles, V., Fuqua, S. A., Jordan, V. C., Katzenellenbogen J. A., Korach, K. S., Maggi, A., Muramatsu, M., Parker, M. G., and Gustafsson, J. A. (2006). International Union of Pharmacology. LXIV. Estrogen Receptors. *Pharmacology Review*. **58**, 773-781.

Vagell, M. E., and McGinnis, M. Y. (1997). The role of aromatization in the restoration of male rat reproductive behavior. *Journal of Neuroendocrinology*. **9**, 415-421.