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ESTROGEN RECEPTOR ALPHA IN THE MEDIAL PREOPIC AREA MEDIATES MALE RAT SEXUAL RESPONSES TO ESTROGEN

by

NANCY RUSSELL

Under the Direction of Andrew N. Clancy, PhD

ABSTRACT

Male rat sexual behavior requires aromatization of testosterone (T) to estradiol (E_2) in the medial preoptic area (MPO) where estrogen receptors (ER) exist in two isoforms, ERα and ERβ. We hypothesized that E_2 acts through estrogen receptor α (ERα) in the MPO to promote male mating behavior. Four groups of male rats were castrated, administered DHT s.c. and bilateral MPO implants delivering either: cholesterol, E_2, propyl pyrazole triol (PPT, ERα agonist), diarylpropionitrile (DPN, ER β agonist), or 1-methyl-4-phenyl pyridinium (MPP, ERα antagonist). Additional gonadally intact males received bilateral MPO DPN implants. PPT maintained sexual behavior equally as well as E_2, whereas mating was not maintained by cholesterol or DPN MPO implants. Exogenous T did not reinstate mating in animals that received MPP MPO implants. These findings indicate that, in the MPO, ERα is necessary and sufficient to promote copulatory behavior in male rats and ERβ is not sufficient for mating.

INDEX WORDS: Medial preoptic area, Estrogen receptor, Estradiol, Dihydrotestosterone, propyl-pyrazole-triol (PPT), Cholesterol, Sexual behavior, Diarylpropionitrile (DPN), 1-methyl-4-phenyl pyridinium (MPP)

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INTRODUCTION

Male rat mating behavior is dependent on testosterone (T), which is enzymatically converted in the brain into estradiol (E_2) by aromatase [Naftolin et al., 1975] and into the non-aromatizable androgen, dihydrotestosterone (DHT) by 5α reductase [Martini, 1982; Massa et al., 1972]. These metabolites, together with T, act on steroid sensitive neurons, including those containing estrogen receptors (ER) in the medial preoptic area (MPO) of the male rat brain to promote mating behavior [Vagell and McGinnis, 1997]. Thus, mating declines after castration and is reinstated by exogenous T [Christensen and Clemens, 1974; Davidson, 1966a, 1966b, 1996]. Futhermore, castrated male rats mate normally (to ejaculation) after being treated with a combination of E_2 and DHT, given in physiological dose ranges, and their mating behavior is comparable to that of rats treated with physiological levels of T [Baum and Vreeburg, 1973; Larsson et al., 1973; Davidson, 1966a, 1966b, 1996]. When administered separately, however, neither E_2 [Baum and Vreeburg , 1973; Davidson, 1969] nor DHT [Feder, 1971; McGinnis and Dreifuss, 1989] fully maintains mating behavior, indicating that estrogens and androgens both are necessary for the normal expression of male rat sexual behavior. Fadrozole, a non-steroidal aromatase inhibitor [Lipton et al., 1990; McGinnis and Mirth, 1986; McGinnis et al., 1996; Vagell and McGinnis, 1997] that blocks formation of E_2 from T, administered either systemically [Bonsall et al., 1992] or intracerebrally into the MPO [Clancy et al., 1995] attenuates male rat mating behavior, an effect that can be partially reversed via administration of E_2 [Bonsall et al., 1992]. Mating is also maintained by implants of E_2 into the MPO of gonadally intact male rats receiving fadrozole s.c. that blocks E_2 formation elsewhere throughout the body and brain [Clancy et al., 2000].

Studies of mating induced expression of Fos immunoreactivity (ir) show that induced Fos-ir occurs in MPO cells that contain receptors for gonadal steroids, including those that express ER-ir [Baum and Everitt, 1992; Coolen et al., 1996; Gréco et al.,1996; Greco et al., 1998; Gréco et al., 2003]. Steroid sensitive cells of the MPO are critical for expression of male rat mating behavior [Brackett and Edwards, 1984; Larsson and Heimer, 1964; Simerly and Swanson, 1986].

Multiple subtypes of ER exist [Greco et al., 2003 ; Kuiper et al., 1996], some of which may be located within the cell membranes of E_2 responsive neurons [Evinger and Levin, 2005; Thomas et al., 2005]. The second type of ER to be discovered was termed ERβ and the original ER is now known as ERα [Kuiper et al., 1996]. ERα and ERβ are both expressed in steroid sensitive neurons of the rat MPO [Abraham et al., 2004; Greco et al., 2003; Nomura et al., 2003, Shughrue and Merchenthaler, 2001]. Evidence from receptor knockout experiments in male mice suggests that ERα may play a dominant role, and ERβ a minor role, in the expression of male sexual behavior [Ogawa et al., 1997; Ogawa et al., 1998; Ogawa et al., 2000; Rissman et al., 1997]. Also, in male rats an anti-sense oilgodeoxynucleotide (AS-ODN), complimentary to ERα mRNA, reduced the expression of ERα-ir when infused bilaterally into the MPO of gonadally intact male rats, which in turn resulted in decreased mating behavior [Paisley, 2007; Paisley et al., 2005]. This suggests that, in the MPO, estrogenic metabolites of T act via ERα, and that ERα is necessary in the MPO for male rat mating behavior to occur.

The recent development of highly selective estrogenic drugs that modulate ERα and/or ERβ function as powerful tools that may aid in identifying the behaviorally relevant ER isoform in the male rat MPO for sexual responses to E_2 [Harrington et al., 2003; Hillisch et al., 2004]. Therefore, in the present study, we used selective estrogenic drugs implanted into the MPO to

test the hypothesis that, in the MPO, ERα mediates sexual responses of male rats to E_2. In two experiments , proven ejaculators were divided into matched groups based on total ejaculation frequency during presurgical sexual behavior tests. In the first experiment, four groups of male rats were castrated and administered DHT s.c. and then received bilateral MPO implants carrying one of the following drugs: i) cholesterol (negative control), ii) E_2 (positive control), iii) propyl pyrazole triol (PPT, an ERα agonist) or iv) diarylpropionitrile (DPN, an ER β agonist). A fifth group was used as a toxicity control that consisted of gonadally intact male rats that also received bilateral MPO DPN implants. In the second experiment, two groups of male rats were castrated, tested until mating behavior declined, and then implanted with T s.c. and bilateral MPO implants delivering either no drugs (blank) or the drug 1-methyl-4-phenyl pyridinium (MPP, an ERα antagonist) [Zhou et al., 2009].

For the first experiment, we predicted that PPT, the ERα agonist, implanted into the MPO would mimic the action of E_2 and fully maintain the mating behavior of castrated, DHT maintained male rats. On the other hand, DPN, an ER β agonist, would not support mating behavior following its implantation into the MPO. Moreover, we predicted that MPO implants of MPP, an ERα antagonist, would block T-induced reinstatement of mating in castrated male rats.

MATERIALS AND METHODS

Animals

Sprague Dawley male and female rats , obtained from Charles River ,were given free access to laboratory chow and tap water and kept in polycarbonate shoebox, 22 x 44 x 18 cm, at Georgia State University. The rats were acclimated to a 14:10 hour reverse light: dark cycle (lights on at 1900 EST). Male rats were housed two per cage until surgery, after which they were housed individually. Females remained paired 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 rat surgical procedures

Females were anesthetized with isoflurane gas in a chamber (5% gas at 1.0 liters per minute oxygen exchange rate) and transferred to a nosecone (2-3% gas at 0.3 liters per minute oxygen). Ovaries were removed through an abdominal incision and a Silastic capsule (6mm length, 1.981 mm i.d. x 3.175 mm o.d.) containing crystalline E_2 was implanted s.c. into the scapular region. Females were allowed at least five days to recover from surgery before being 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.

Sexual behavior tests

Male rats were paired with sexually receptive females and screened once weekly for three consecutive weeks for the display of sexual behavior prior to being selected for the experiment. Tests for sexual behavior were conducted by blind observers once a week, 30 minutes per test. Sexual behavior testing took place under red light illumination during the dark phase of the light:

dark cycle. The recorded behaviors during each 30 minute test included: mount frequency (MF): number of mounts with no penetration; intromission frequency (IF): numbers of mounts 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. The same sex behavior testing procedures were used after males had recovered from surgery.

Experiment 1

Male rat experimental groups and surgery

Upon completion of sexual screening trials, four groups of male rats, all proven ejaculators, were matched and divided into groups, according to total numbers of ejaculations during sexual screening trials, then males were castrated, administered DHT s.c., and received bilateral MPO implants carrying one of the following drugs: i) cholesterol (negative control), ii) E_2 (positive control), iii) PPT (ERα agonist) or iv) DPN (ERβ agonist). A fifth matched group of gonadally intact male rats served as DPN toxicity controls; these animals were not castrated but received bilateral MPO implants of DPN that were identical to those used in the DPN group(see above iv). Specifically, males were anesthetized with isoflurane gas in a chamber and transferred to a nosecone as described above for females, castrated through midline abdominal incisions, and received s.c. in the scapular region a 10-mm Silastic capsule (1.981 mm ID x 3.175 mm OD) containing crystalline DHT. DHT capsules of these dimensions have been reported to produce circulating DHT levels in the physiological range [Ando et al., 1998; Lugg et al., 1995; Parte and Juneja, 1992]. They were then placed in a stereotaxic instrument and

implanted bilaterally with ethylene oxide sterilized 22 gauge stainless steel guide cannulae (Plastics One, Roanoke, VA, USA) aimed at the MPO (level skull coordinates with respect to bregma: anterior-posterior = -0.5 mm, medial-lateral = ± 0.75 mm, dorsal-ventral = -8.0 mm [Swanson, 1998]. Males serving as gonadally intact DPN toxicity controls were treated identically except they were neither castrated nor given DHT. Sterile, 28 gauge stainless steel inner cannulae, extended 1 mm below the guide cannulae; these were tamped in either E_2, DPN, PPT, or cholesterol to deliver the appropriate drug into the MPO. Thereafter, rats were allowed at least five days to recover from surgery before 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 ensure drug was present, none failed.

Experiment 2

Male rat experimental groups and surgery

MPP (ERα antagonist) or blank (control) drug carrying MPO cannulae were implanted in castrated male rats that were administered T exogenously to test if MPP would interfere with T-induced reinstatement of mating. Specifically, male rats were screened for sexual behavior and proven ejaculators were matched on total numbers of ejaculations. Thereafter the males were anesthetized as described previously and castrated through midline abdominal incisions. After a recovery period of at least five days, the castrated males were tested with receptive females once a week until all mating ceased. Each male was then anesthetized as described before and implanted s.c. with a 15 mm Silastic capsule (1.981 mm ID x 3.175 mm OD) containing crystalline T. T capsules of this size have been reported to produce circulating T levels in the physiological range [Verjans et.al., 1975]. Immediately thereafter, they were stereotaxically

implanted bilaterally with 22-gauge stainless-steel, ethylene oxide-sterilized, guide cannulae (Plastics One, Roanoke, VA, USA) aimed at the MPO (level skull coordinates with respect to bregma: anterior-posterior = –0.5 mm, medial-lateral = ± 0.75 mm, dorsal-ventral = –8.0 mm [Swanson, 1998]. Sterile stainless steel inner cannulae (28 gauge, Plastics One) extended 1 mm below the guide cannulae and were tamped in either: (i) MPP or (ii) nothing (blank). Males were given 5–7 days to recover before the weekly postoperative behavioral testing resumed. Freshly tamped sterilized inner cannulae were inserted, under brief isoflurane anesthesia, into the brain 48 h before and 24 h after every behavioral test to ensure continuous drug delivery to the brain. Tips of cannulae were examined microscopically after they were removed from the brain to ensure drug was present, none failed.

Histological Verification

When the testing period was concluded, males were euthanized with an overdose of sodium pentobarbital (1.0 mg/kg i.p.) and perfused transcardially for 5 min with physiological saline followed by a minimum of 300 ml of a fixative containing 2% paraformaldehyde and 0.1% picric acid in 0.1 M phosphate buffer (PB) 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 approximately 48 h. Frozen coronal brain sections (40 μm) through the diencephalon were collected into 0.1 M PB, mounted on clean gelatin albumin-coated slides, stained with toluidine blue and examined microscopically to verify cannula placement.

Statistical analysis

In the first experiment, between-group behavioral data were analyzed by repeated measures (group by trial) analysis of variance for the pre-operative trials (5 groups by 3 trials)

and for the post operative trials (5 groups by 4 trials), followed by *post hoc* comparisons using the Tukey honestly significant differences test at a probability level of 0.05 [Kirk, 1968]. Within-group comparisons of preoperative and postoperative changes in behavior were then analyzed by paired t-tests [Kirk, 1968]. In the second experiment, group differences were analyzed by the Mann-Whitney U test [Siegel, 1956]. 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

Experiment 1

Male sexual behavior

The mating behavior of sexually experienced, castrated male rats receiving DHT s.c. was maintained by either E_2 or PPT implants to the MPO, but neither cholesterol nor DPN MPO implants maintained mating (FIGURE 1). Gonadally intact DPN toxicity controls that received DPN MPO implants also mated robustly. Specifically, the two groups receiving either E_2 or PPT MPO implants and gonadally intact DPN toxicity controls mated vigorously on all sexual behavior tests throughout the study and these three groups were statistically indistinguishable from each other on all measures of copulatory behavior during both the preoperative and postoperative periods; sexual behavior did not change significantly within these three groups before vs. after treatment. In contrast, sexually experienced, castrated, DHT s.c. treated males that received either cholesterol or DPN implants into the MPO virtually ceased mating during the postoperative period whereas they had mated robustly prior to surgery. It is noteworthy that, during the preoperative period, all five groups mated vigorously and no group differences were

observed. Thus, MPO implants of E_2 itself or PPT (ERα agonist) but not cholesterol or DPN (ERβ agonist) maintained mating behavior.

There were no significant group differences on any of the six measures of copulatory behavior during the preoperative trials, nor were there any significant interactions. Significant behavioral differences emerged, however, during the postoperative period (FIGURE 1). Specifically, during the postoperative period, the groups differed significantly from each other on mount frequency ($F_{4,36} = 11.42$, $P < 0.001$), ejaculation frequency ($F_{4,36} = 9.79$, $P < 0.001$), intromission frequency ($F_{4,36} = 19.31$, $P < 0.001$), mount latency ($F_{4,36} = 13.09$, $P < 0.001$), ejaculation latency ($F_{4,36} = 5.52$, $P < 0.002$), and the post-ejaculatory interval ($F_{4,36} = 9.23$, $P < 0.001$). No other statistically significant main effects or interactions were observed during the postoperative period. Follow-up post-hoc analysis of sexual behavior during the postoperative period revealed no significant differences on any test day between the E_2 group compared to either the PPT or gonadally intact DPN toxicity control groups on any of the six behavioral measures all of which groups continued to mate vigorously, except that, on the first postoperative trial, rats receiving MPO E_2 implants ejaculated significantly more frequently than those that received MPO PPT implants ($P < 0.05$). In contrast, the E_2 group mated significantly ($P < 0.05$) more than the cholesterol group on all postoperative test days and on all six behavioral measures except mount frequency on the first postoperative trial, ejaculation frequency on the fourth postoperative trial, ejaculation latency on the first and fourth postoperative trials and the post-ejaculatory interval on the first postoperative trial. Similarly, the E_2 group mated significantly ($P < 0.05$) more than the DPN group on all postoperative test days and on all six behavioral measures except mount frequency on the first and fourth postoperative trials and mount latency, ejaculation latency and the post-ejaculatory interval on the first postoperative trial. In addition,

the cholesterol group mated significantly ($P < 0.05$) less than the PPT group on all postoperative test days and on all six behavioral measures except mount frequency, ejaculation frequency and ejaculation latency on the first and fourth postoperative trials and the post-ejaculatory interval on the first post operative trial, whereas the cholesterol group mated significantly ($P < 0.05$) less than the gonadally intact DPN toxicity control group on all postoperative test days and on all six behavioral measures except ejaculation frequency on the second postoperative trial, ejaculation latency on the first, second and fourth postoperative trials and the post-ejaculatory interval on the first postoperative trial. Finally, the cholesterol group differed significantly ($P < 0.05$) from the DPN group only on ejaculation latency on the fourth postoperative trial.

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 six indices of copulatory behavior in gonadally intact DPN toxicity controls, nor did the groups of castrated males maintained on DHT s.c. that received MPO implants of either E_2 or PPT differ significantly on any behavioral measure before and after surgery. However, compared to their preoperative mating performances, sexual behavior deteriorated significantly during the postoperative period in the groups of castrated males maintained on DHT s.c. and implanted in the MPO with either cholesterol or DPN. Specifically, in the cholesterol group, mount frequency ($t = 6.21$, $P < 0.001$) and intromission frequency ($t = 10.32$, $P < 0.001$) were significantly depressed on the last postoperative test compared with the last preoperative day (FIGURE 1), whereas mount latency ($t = 3.95$, $P < 0.003$) and the post-ejaculatory interval ($t = 3.03$, $P < 0.01$) were significantly longer on the last postoperative test relative to the last preoperative day. Similarly, in castrated, DHT s.c.-maintained male rats receiving DPN MPO implants, ejaculation frequency ($t = 4.67$, $P < 0.003$)

and intromission frequency ($t = 5.73$, $P < 0.001$) were significantly depressed on the last postoperative test compared with the last preoperative day (FIGURE 1), whereas mount latency ($t = 3.86$, $P < 0.008$), ejaculation latency ($t = 5.86$, $P < 0.001$) and the post-ejaculatory interval ($t = 3.82$, $P < 0.009$) were significantly longer on the last postoperative test relative to the last preoperative day.

Cannulae Placements

Locations of cannula tips are shown in FIGURE 2. Cannula tips were located in or near the MPO or anterior hypothalamus for all males

Experiment 2

Male sexual behavior

Exogenous T rapidly reinstated mating behavior in control males that received blank MPO implants. In contrast, exogenous T did not promote significant restoration of copulatory behavior in animals that received MPP MPO implants, indicating MPP interfered with T-induced reinstatement of sexual behavior. Specifically, on trial 2, rats receiving blank MPO implants mounted ($U_{(n1=5,\ n2=4)} = 0$, $p < 0.014$) and intromitted ($U_{(n1=5,\ n2=4)} = 0$, $p < 0.014$) significantly more frequently than animals that received MPP MPO implants and, on trial 2, there was a non-significant trend toward greater ejaculation frequency in the blank MPO groups than in the MPP MPO group ($U_{(n1=5,\ n2=4)} = 2.5$, $p < 0.066$ = ns), TABLE 1. It is noteworthy that, by the second trial, all males that had received blank MPO implants mounted and intromitted and three of these four rats mated to ejaculation. In contrast only one of the five males in the MPP MPO group mounted and none intromitted or ejaculated. Mount latency, Ejaculation Latency and PEI data are not shown.

Cannulae Placements

Locations of cannula tips are shown in FIGURE 3. Cannula tips were located in the MPO or anterior hypothalamus for all males.

TABLE 1.1 Statistical Analysis MPP Experiment

GROUP:	**BLANK to MPO (N=4)**		**MPP to MPO (N=5)**	
BEHAVIOR:	Trial 1	Trial 2	Trial 1	Trial 2
Mount Frequency	15.25 ± 8.65	*22.25 ± 3.59 *p < 0.014 *Blank vs MPP, Trial 2 MF	1.2 ± 1.2	*0.2 ± 0.2
Intromission Frequency	11.75 ± 7.15	*14.25 ± 5.02 *p < 0.014 *Blank vs MPP, Trial 2 IF	0 ± 0	*0 ± 0
Ejaculation Frequency	0.75 ± 0.48	1.5 ± 0.5	0 ± 0	0 ± 0

DISCUSSION

The objective of these experiments was to test the hypothesis that, in the MPO, ERα mediates the sexual responses of male rats to E_2. This hypothesis was confirmed by examination of the copulatory response of animals implanted in the MPO with various selective ERα or ERβ agonists or antagonists. We found that, in the MPO, ERα alone is necessary and sufficient to promote mating behavior. Specifically, the mating behavior of sexually experienced, castrated male rats receiving DHT s.c. was fully maintained by MPO implants of either E_2 or PPT (ERα agonist) but neither MPO implants of DPN (ERβ agonist) nor cholesterol were effective in maintaining copulatory behavior. Because the group of gonadally intact males with DPN MPO implants (DPN toxicity controls) mated vigorously on all sexual behavior tests before and after

surgery and did not differ significantly from the two groups receiving either E_2 or PPT MPO implants, it is unlikely that DPN is toxic to the brain, nor does it interfere with mating. Moreover, MPO implants of MPP (ERα antagonist) inhibited T-induced reinstatement of sexual behavior, which extends and replicates, by an independent method, the findings in a companion report that blocking the synthesis and expression of ERα in the MPO reduces sexual behavior [Paisley, 2007; Paisley et al., 2005]. It is unlikely that MPP is toxic to the brain or interferes non-specifically with mating because in other experiments in our laboratory [Ogaga-Mgbonyebi et al., 2009], MPP was implanted into the amygdala of gonadally intact male rats, which animals continued to mate vigorously. This pattern of results indicates that, in the MPO, ERα is critical for mating. Taken together these findings support the interpretation that, in the MPO, ERα exclusively mediates sexual responses to E_2 and, in this brain area. ERα is necessary and sufficient to promote mating behavior in male rats, whereas, in the MPO, ERβ is not sufficient for the expression of this behavior.

In this study we report on the effects on male rats' copulatory behavior of the selective ER agonists PPT and DPN and antagonist MPP following their direct, site-specific implantation to the brain. Previous studies have reported only in female rats, that received intraventricular application of PPT but not DPN, which promoted lordosis, presumably by action on the ventromedial hypothalamus [Mazzuco et al.,2008; Walf and Frye, 2005; Walf et al., 2007]. In male rats, steroid sensitive MPO neurons expressing ER alone, or androgen receptors (AR) only, or that co-express both AR and ER are activated by mating [Greco et al., 1998;]. However, ER exists in at least two isoforms, ERα and ERβ [Kuiper et al., 1996]. Knockout (KO) studies in male mice suggest that the form of ER that is centrally important for display of male sexual behavior is ERα [Ogawa et al., 1997; Ogawa et al., 1998; Ogawa et al., 2000; Rissman et al.,

1997]. Nevertheless, whereas ERα KO profoundly reduced mating [Ogawa et al., 1997, Ogawa et al., 1998] and ERβ KO showed only a minimal reduction [Scordalakes et al., 2003], the combined knockouts of ERα and ERβ [Owaga et al., 2000] virtually eliminated mating, suggesting a major role for ERα and a minor role for ERβ in male mouse mating. Several other laboratories [Davis and Barfield, 1979; Greco et al.,1998; Greco et al., 2003; Walf et al., 2007] have shown that ERα or ERβ mediate various reproductive and non-reproductive behaviors by their actions in the brain. In female rats, ERα-ir and ERβ-ir neurons in MPO and MEA were both activated by sexual behavior and expressed mating-induced Fos-ir, raising the possibility that both ER subtypes make a contribution to sexual behavior [Greco et al., 2003]. Previous findings from our laboratory show that estrogenic metabolites of T act in the MPO to promote mating behavior in male rats [Clancy et al., 1995, 2000]. These findings were extended by experiments with gonadally intact male rats in which an anti-sense oligodeoxynucleotide (AS-ODN) complimentary to ERα mRNA was infused into the MPO, which reduced significantly the expression of ERα-ir in the MPO and resulted in significantly decreased mating behavior [Paisley, 2007; Paisley et al., 2005]. These findings, together with those in the present study, suggest ERα-related signal transduction pathways in the MPO are important in mediating male rat sexual responses to E_2 but that neither ERβ/ERβ homodimerization nor ERα/ERβ heterodimerization in the MPO are necessary for mating. Moreover, MPO implants of either E_2 conjugated to bovine serum albumin (BSA-E_2) or unconjugated (free) E_2 were equally effective in maintaining male rat mating behavior [Huddleston et al., 2006], suggesting the possible existence in the MPO of an ERα signal transduction pathway that is mediated by a plasma membrane bound version of ERα (mERα). This is because BSA-E_2 is believed to prevent the entry of E_2 into the interior of target cells thereby confining its action to the cell surface

[Huddleston et al., 2006; Pappas et al., 1995]. Therefore, a novel mechanism of E_2 action may exist in the MPO in which an mERα influences the sexual behavioral responses to E_2. If such a mERα MPO signal transduction pathway exists, then it may be possible for the MPO to be quickly and easily activated whenever the social or environmental circumstances are ideal for mating behavior to be expressed [Abraham et al., 2004; Chaban et al., 2004; Pietras and Szego, 1977]. Stimulus-induced fluctuations in levels of circulating gonadal steroids such as female-induced or pheromonally-induced elevations in gonadotropins and T [Clancy et al., 1984, Coquelin et al., 1984, Khan et al., 2005; Macrides et al., 1975], if accompanied by rapid aromatization of T to E_2, conceivably could activate the MPO via a mERα to promote rapidly the expression of male mating behavior.

Different ligands such as PPT, DPN, MPP or E_2 bind selectivity to different ER subpopulations thereby eliciting different responses [Staufer et al., 2000; Wright et al., 2006]. Cholesterol presumably is inert and does not activate either ERα or ERβ, thus after cholesterol MPO implantation, mating ceased. In contrast, PPT binds ERα with a high affinity and exhibits limited cross reactivity with ERβ [Stauffer et al., 2000] whereas DPN binds ERβ robustly and cross reacts weakly with ERα [Katzenellenbogen et al., 2000], and E_2 activates both ERα and ERβ and promotes male rat mating behavior. MPO implants of MPP, interfered with T-induced reinstatement of mating consistent with previous reports MPP is a selective ERα antagonist that has high affinity for ERα and little cross reactivity for ERβ [Sun et al., 2002; Zhou et al., 2009]. These characteristics of the selective estrogenic drugs used in this study further suggest that, in the MPO, ERα mediates responses to E_2 to promote sexual behavior. Taken together, these findings indicate that, in the MPO, ERα is necessary and sufficient to mediate male rat sexual behavioral responses to E_2.

The central findings from other studies in our laboratory [Paisley, 2007; Paisley et al., 2005, Ogaga et al.,2009] indicate, however, that E_2 acts differently in the MPO than in the MEA and there may be a role for ERβ to play, outside the MPO, in the expression of male mating behavior. Thus, many questions remain how these ER subtypes promote expression of copulatory responses. For example, either ERα or ERβ may simply be the most numerous ER subtype in any given brain region and thus govern the overall response of that brain region to E_2. Although previous findings [Greco et al., 1998; Greco et al., 2003] suggest it is unlikely that the different ER subtypes are each selectively associated with the expression of specific behaviors, [Toufexis et al., 2007] addressed the possibility that the ambiguous effects of E_2 in the MEA may result from mutually opposing interactions between ERα and ERβ at the cellular level. If this is case, then differences in the ERα/ERβ ratios in different brain regions might influence the response of any given brain area to E_2. Based on the findings in this report, we infer that, in the MPO of castrated male rats receiving DHT s.c., E_2 mediates mating via ERα and that this pathway alone is necessary and sufficient to activate male sexual behavior.

In conclusion, this study provides evidence suggesting that, in the MPO, ERα is the behaviorally relevant form of ER mediating male rat mating behavior. Thus, following MPO implantation of PPT, males mated robustly at rates that were statistically indistinguishable from those implanted in the MPO with E_2. The effects of any ligand, such as E_2, are mediated by the specific receptors that bind them. Once bound, there are at least two ways that E_2 may affect its targets, either by activating the traditional genomic pathway [Jensen et al., 1968; King and Greene, 1984] or via a rapid non-genomic pathway that initiates synthesis of intracellular second messengers [Minami et al., 1990; Pietras and Szego, 1977, 1980]. Further investigation is needed to determine the mechanism of action of MPO ERα. It is noteworthy, however, that in

the MPO, BSA-E_2, which is believed not to penetrate into the interior of cells, supported vigorous mating behavior [Huddleston et al., 2006], which raises the possibility that a plasma membrane-associated ERα in neurons of the MPO mediates male rat sexual responses to E_2. From the present study, it may be concluded that, in the MPO, ERα alone is necessary and sufficient to maintain mating in castrated, androgen supplemented male rats.

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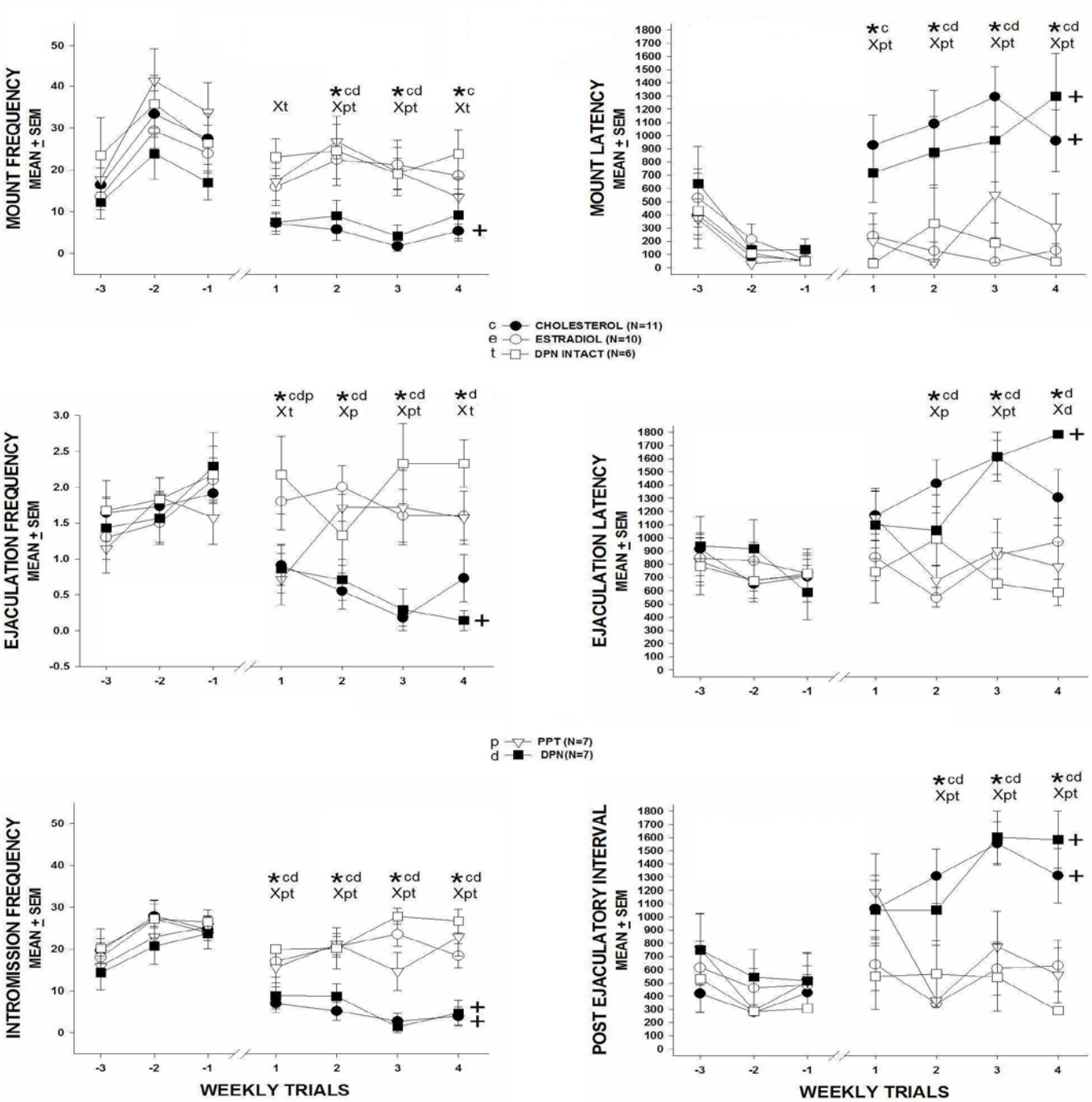
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FIGURE 1: MALE SEXUAL BEHAVIOR. Male rats receiving PPT implants to the MPO (p) maintained high levels of mating behavior and did not differ significantly from rats receiving MPO E_2 implants (e). Animals receiving cholesterol MPO implants (c), however, exhibited significantly lower levels of sexual behavior than either of these two groups. DPN did not promote mating in castrated, DHT-maintained males (d) but gonadally intact (DPN toxicity controls) rats (+) mated normally showing that DPN is not toxic.

* Between group difference from estrogen, $p<0.05$.

X Between group difference from cholesterol, $p<0.05$.

+ Within group difference from last preoperative sexual behavior test, $p<0.05$.

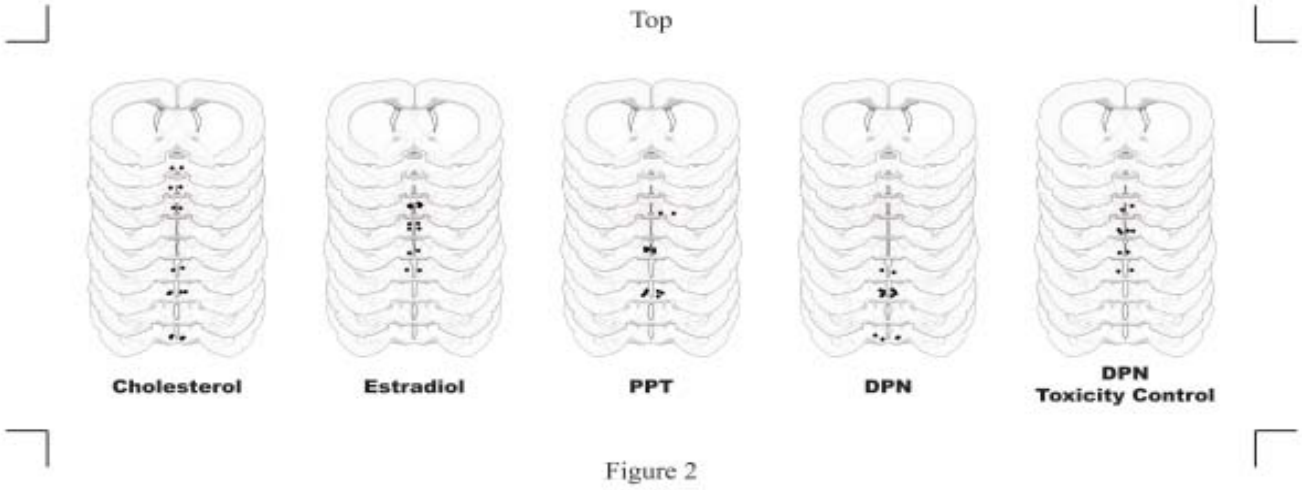

FIGURE 2: EXPERIMENT 1

CANNULA PLACEMENTS TO THE MPO. Cannula tips were located in the MPO or anterior hypothalamus for all males.

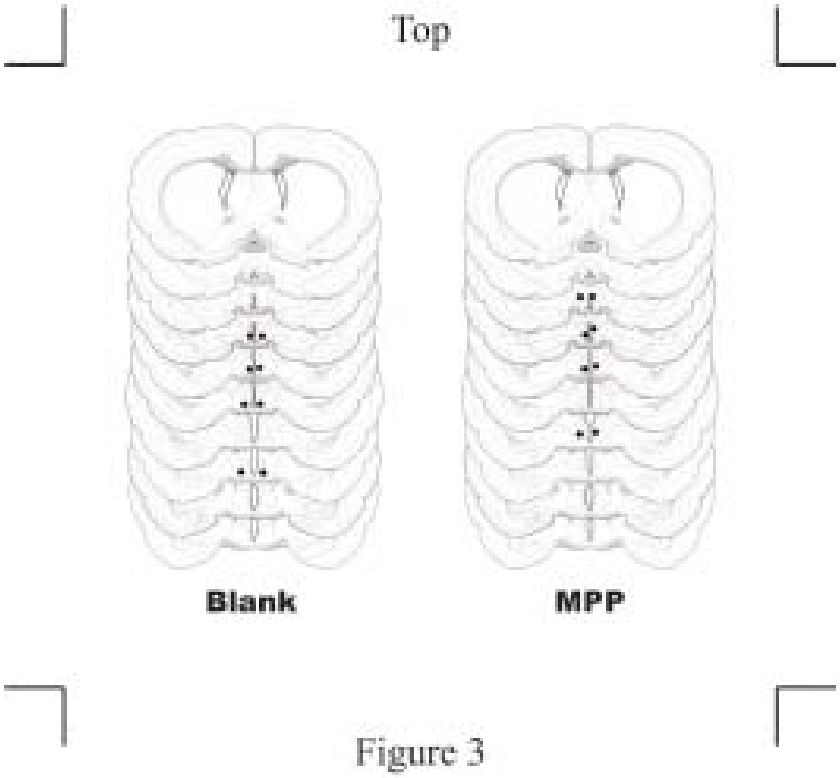

FIGURE 3: EXPERIMENT 2, CANNULA PLACEMENTS TO THE MPO. Cannula tips were located in the MPO or anterior hypothalamus for all males.