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Effects of testosterone metabolites on copulation and medial preoptic dopamine release in castrated male rats

Susan K. Putnam,[1] Satoru Sato, and Elaine M. Hull*

Department of Psychology, University at Buffalo, State University of New York, Buffalo, NY 14260-4110, USA

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Abstract

The medial preoptic area (MPOA) is an important integrative site for male sexual behavior. Dopamine (DA) is released in the MPOA of male rats shortly before and during copulation. The recent presence of testosterone (T) may be necessary for this precopulatory increase in release. Previously, the postcastration loss of copulatory ability mirrored the loss of the DA response to an estrous female, and the restoration of copulation with exogenous T was concurrent with the reemergence of this DA response. The present study investigated the effectiveness of the two major metabolites of T in maintaining copulation and basal and female-stimulated DA levels. Adult male rats were castrated and received daily injections of estradiol benzoate (EB), dihydrotestosterone benzoate (DHTB), EB + DHTB, testosterone propionate (TP), or oil vehicle for 3 weeks. Microdialysis samples were collected from the MPOA during baseline conditions, exposure to an estrous female behind a barrier, and copulation testing. EB + DHTB- and TP-treated animals had normal basal DA levels and showed a precopulatory DA response, and most copulated normally. EB-treated castrates had high basal DA levels, but failed to show a female-stimulated increase; most intromitted, but none ejaculated. DHTB- and oil-treated groups had low basal levels of extracellular DA that did not increase during copulation testing; most failed to mount and none ejaculated. These results suggest that E maintains normal basal levels of extracellular DA in the MPOA, which are sufficient for suboptimal copulation, but that androgen is required for the female-stimulated increase in DA release and for facilitation of ejaculation.

Keywords: dopamine; medial preoptic area; copulation; testosterone; dihydrotestosterone; estrogen; microdialysis; HPLC-EC; male rats

Introduction

The medial preoptic area (MPOA) is a critical integrative site for male sexual behavior in virtually all vertebrate species, including the rat (reviewed in Hull et al., 2002). MPOA lesions impair sexual behavior (Heimer and Larsson, 1966/67; Giantonio et al., 1970; de Jonge et al., 1989; Liu et al., 1997), whereas stimulation of the MPOA enhances behavior (Malsbury, 1971; Paredes et al., 1990; Rodriguez-Manzo et al., 2000).

The neurotransmitter dopamine (DA) is also important for sexual behavior in numerous species (reviewed in Bitran and Hull, 1987; Melis and Argiolas, 1995). Stimulation of DA receptors in the MPOA facilitates copulation, sexual motivation, and genital reflexes in male rats (reviewed in Hull, 1995; Hull et al., 1999, 2002). We have observed a consistent relationship between MPOA DA release during precopulatory exposure to a receptive female behind a barrier and the ability of the male to copulate after removal of the barrier (Hull et al., 1995; Putnam et al., 2001).

Chronic systemic or intracranial administration of testosterone propionate (TP) maintains or restores copulatory ability in castrated male rats (Beach and Holtz-Tucker, 1949; Davidson, 1966, 1969; Hawkins et al., 1988; McGinnis et al., 1989; Roselli and Chambers, 1999). Systemically administered T is also permissive for the MPOA DA response to an estrous female. Most vehicle-treated males that were castrated 1 week previously showed the precopulatory

* Corresponding author. Fax: +1-716-645-3801.
E-mail address: emhull@acsu.buffalo.edu (E.M. Hull).
[1] Current address: Department of Psychology, Canisius College, Buffalo, NY 14214.

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DA response and copulated after removal of the barrier (Hull et al., 1995). The remainder of 1-week castrates, and all 2-week castrates, failed to show the precopulatory DA response and failed to copulate. T injections maintained both the DA response and copulation. More recently, 5 days was a threshold period for testosterone's restoration of copulation and the MPOA DA response in long-term castrates (Putnam et al., 2001). Thus, restoration of copulatory ability by T in long-term castrates mirrored the restoration of the MPOA DA response to an estrous female.

T is primarily a prohormone that can be aromatized to estradiol (E) or reduced to 5α-dihydrotestosterone (DHT) in various tissues, including the brain. These metabolites regulate transcription of different sets of genes (reviewed in McGinnis et al., 2002; Pfaff et al., 2002). Unlike T, DHT cannot be aromatized to E. DHT, administered alone to castrated male rats, is typically ineffective in maintaining or restoring copulation (McDonald et al., 1970; Baum and Vreeburg, 1973; Beyer et al., 1973; Feder et al., 1974; Hawkins et al., 1988). E, administered alone, produces variable results, but is typically less effective than T (Baum and Vreeburg, 1973; Larsson et al., 1973; Feder et al., 1974; Lodder and Baum, 1977; Hawkins et al., 1988; Roselli and Chambers, 1999). However, a regimen of combined E + DHT is the most efficacious in maintaining full copulatory behavior in castrated male rats (Baum and Vreeburg, 1973; Larsson et al., 1973; Feder et al., 1974; Lodder and Baum, 1977; Hawkins et al., 1988). Such observations suggest that both androgenic and estrogenic metabolites make important contributions to male rat sexual behavior. T, E, and DHT may differ in their capacities to maintain sexual behavior, in part, because they have different potencies in promoting an increase in MPOA DA release.

The present study used in vivo microdialysis and HPLC with electrochemical detection (HPLC-EC) to investigate whether E, DHT, or the combination of the two is sufficient to maintain basal extracellular DA levels in the MPOA, a DA response to an estrous female, and/or copulation.

Materials and methods

Subjects

Adult male Long–Evans Blue-Spruce rats (250–300 g) obtained from Harlan Sprague Dawley (Indianapolis, IN) were individually housed in clear plastic cages in a temperature- and humidity-controlled environment with food and water available ad libitum. They were maintained on a 14:10 reversed light cycle, with lights off at 11:00 h, and weighed daily to monitor health status and to accustom them to handling. All animals were screened for copulatory ability 1 week after arrival, using stimulus females of the same strain.

Stimulus females

Female rats were ovariectomied under ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4 mg/kg) anesthesia. They were brought into estrus with subcutaneous injections of estradiol benzoate (EB) (20 μg) (Sigma Chemicals, St. Louis, MO) 48 hr, prior to testing and progesterone (500 μg) (Sigma) 4 h prior to testing. Behavioral receptivity of each female was verified before copulation testing by permitting three intromissions by a stud male in his home cage.

Copulation screening of test males

For copulation screening of the test males, a proven estrous female was placed in the home cage of each subject for 60 min, during which time mounts, intromissions, and ejaculations were recorded. Testing was considered complete when two ejaculations were achieved. If after four sessions an animal had not ejaculated twice, it was not used for this study.

Surgery

After copulation testing, all sexually active male rats were anesthetized with an i.m. injection of ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4 mg/kg) and castrated. A longitudinal midscrotal incision was made, and the testes were tied off and removed with a cut distal to the ligature. Bacitracin ointment was applied to the incision site to prevent infection. Each subject also received a subcutaneous injection of the antibiotic Gentamicin (0.03 mg/kg) immediately after surgery and was weighed and checked daily for general health.

One week after orchidectomy, each subject was deeply anesthetized with ibid. ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4 mg/kg) and placed into a Kopf stereotaxic frame with the incisor bar set at +5 mm. All rats were implanted with a 15-mm, 23-ga thin-wall stainless steel guide cannula, ending 2 mm above the left MPOA (mm from bregma: AP, +2.2; ML, +0.2; DV, −6.2; incisor bar, +5). A metal male electrical clip was adhered to the top of the skull near the cannula with dental cement and was used to attach the animal to swivel-mounted microdialysis equipment on the day of testing. This assured stability of the probe assembly while simultaneously permitting the male to move freely and to interact with the female during behavioral testing. An obturator made from 27-ga stainless steel tubing and cut to the length of the guide cannula was inserted to keep the cannula free of debris. Each subject received an injection ibid. of Gentamicin (0.03 mg/kg) and was monitored closely for any post-surgical complications. None of the animals experienced serious weight loss (>20 g) or showed any other signs of illness following either castration or intracranial surgery. (Details of cannula construction are described in Hull et al.,

1986). All procedures were in accordance with the National Institutes of Health Guidelines for the Use of Animals and were approved by the local Institutional Animal Care and Use Committee.

Hormone replacement

Animals were randomly assigned to one of four hormone treatment groups or to a control (oil vehicle) group. Each received daily subcutaneous injections of 20 μg EB, 500 μg dihydrotestosterone benzoate (DHTB) (Sigma), a combination of 20 μg EB and 500 μg DHTB, or 500 μg testosterone propionate (TP) (Sigma) in 0.1 ml olive oil, or with 0.1 ml oil for the control group, for a period of 21 days beginning the day after castration. These hormone doses are supraphysiological, but were chosen because they were maximally effective in maintaining or restoring copulation in castrated male rats (Feder et al., 1973; Larsson et al., 1973).

Microdialysis

Microdialysis probes using a concentric flow design were used. A 3-mm length of dialysis membrane (MW cutoff 12,000; 210 μm o.d.; Spectra-Por) was glued to one end of a 27-ga 15-mm stainless steel shaft with waterproof epoxy. The end was plugged and 2 mm was inactivated with epoxy to maintain an active dialyzing length of 1 mm. Samples were collected at 6-min intervals into 250-μl centrifuge tubes and immediately frozen in dry ice, followed by storage in a −80°C supercool freezer.

On the day of testing, subjects were lightly anesthetized with ketamine hydrochloride (12.5 mg/kg) and xylazine hydrochloride (1 mg/kg) to facilitate insertion of the microdialysis probe. Flow of the perfusion medium (0.5 μl/min) began immediately after probe insertion. A stabilization period of 4 h was permitted between insertion of the probe and collection of dialysate samples.

Following the stabilization period, dialysate samples were collected every 6 min for 1 h (10 baseline samples). At that time, a receptive female was placed into a metal cage suspended over the male's home cage, permitting olfactory, visual, and auditory stimulation but not direct physical interaction. Four 6-min estrous-female samples were collected during this phase of testing. The female was then placed into the male's home cage, and the animals were allowed to copulate for 30 min. Five 6-min copulatory samples were collected during this time, and behavioral parameters were recorded. (Two min were permitted to pass between collection of estrous-female and copulation samples to allow for dead volume in the probe.)

Behavioral testing

During behavioral testing on the day of dialysate collection, the following measures were recorded: mount latency (ML), latency from the introduction of the female to the first mount; intromission latency (IL), latency from introduction of the female to the first intromission; ejaculation latency (EL), latency from the first intromission to ejaculation; postejaculatory interval (PEI), latency from the first ejaculation to the first intromission of the second ejaculatory series; mount frequency (MF), number of mounts without vaginal insertion during the 30-min test period; (IF), number of mounts with penile insertion during the 30-min test period; ejaculation frequency (EF), number of ejaculations per 30-min test. For animals that ejaculated, the number of intromissions preceding ejaculation was also recorded (IF_1). Percentages of each group that exhibited mounts, intromissions, and ejaculations were also analyzed. Latencies were analyzed only for animals that performed the relevant behavior.

HPLC-EC

Dialysate DA was assayed using HPLC-EC. Samples were loaded via a Rheodyne injector valve, which delivered a 500-nl volume to an LC-Packings Fusica C18 capillary column. Mobile phase, consisting of 30 mM citric acid, 50 mM sodium acetate, 0.027 mM Na_2EDTA, 0.25 mM octyl sodium sulfate, and 2.5% acetonitrile (degassed and pH 3.8), was delivered by a Gilson Model No. 307 pump operating at 0.5 ml/min. The Gilson pump was equipped with an Acurate flow splitter, which delivered 6 μl/min to the column. Compounds were detected with an Antec microcell detector, using a glassy carbon working electrode maintained at a potential of +0.7 V relative to a Ag/AgCl reference electrode.

Evaluation of HPLC data

Daily calibration curves based on known amounts of external standards were established each day of sample injection and assay. Using a Gilson Unipoint program, the amount of DA present in the final three samples collected before introduction of the female, and all samples collected thereafter, was evaluated and expressed in picograms per microliter of dialysate.

Histology

Immediately after copulation testing and final dialysate collection, each rat was overdosed with sodium pentobarbital, and cresyl violet dye was perfused through his microdialysis probe. Subjects were then decapitated, and brains were removed for histological verification of probe placement. Only those animals with blue dye in the region of the MPOA were used for statistical analyses.

Table 1
Effects of hormone treatments on copulatory behavior

Hormone condition	ML (s)	MF	IL (s)	IF	EL (s)	EF	PEI (s)
Oil	NA	0.3 ± 0.3[a]	NA	0.0 ± 00[a]	NA	0.0 ± 0.0	NA
$n = 4$	1/4		0/4		0/4		0/4
DHTB	NA	3.2 ± 3.2	NA	0.4 ± 0.4[a]	NA	0.0 ± 0.0[b]	NA
$n = 5$	1/5		1/5		0/5		0/5
EB	22.1 ± 0.9	17.0 ± 3.0	252.9 ± 119.9	6.9 ± 0.9	NA	0.0 ± 0.0[b]	NA
$n = 7$	7/7		7/7		0/7		0/7
EB+DHTB	14.6 ± 2.8	14.2 ± 2.1	339.6 ± 280.9	8.8 ± 3.0	746.5 ± 282.3	0.6 ± 0.2	590.8 ± 224.4
$n = 5$	5/5		5/5		3/5		3/5
TP	17.8 ± 4.8	5.8 ± 0.5[a]	129.8 ± 29.4	11.8 ± 3.4	611.5 ± 45.0	1.3 ± 0.3	604.2 ± 35.9
$n = 4$	4/4		4/4		4/4		4/4

Note. Means ± SE for each observed behavioral parameter during 30-min copulation test. ML, latency to mount; MF, number of mounts during 30-min test; IL, latency to intromit; IF, number of intromissions during 30-min test; EL, latency to ejaculate; EF, number of ejaculations during 30-min test; PEI, time between ejaculation and the next intromission. The number of animals in each treatment group that displayed the behavior of interest is designated by the ratio in the latency cell. NA, not analyzed because too few animals performed the behavior.

[a] Significantly different from EB-tretaed group.

[b] Significantly different from TP-treated group.

Statistical analyses

Data from 25 animals that had less than 10% variation among the last three baseline samples, had correct probe placements, and from which dialysate samples were successfully obtained were included in statistical analyses. Frequencies of copulatory behaviors were compared among all hormone treatment groups with Kruskall–Wallis tests, followed by Mann–Whitney *U* tests, using Bonferroni's correction for multiple comparisons. Latency and duration measures and IF_1 data were analyzed by one-way ANOVAs or *t* tests, including only the animals that exhibited the behaviors. Chi-square tests were used to analyze the percentage of each group showing each behavior. DA data were analyzed using a two-way mixed ANOVA for hormone condition × sample period as a repeated factor. All significant treatment effects and interactions were further analyzed with appropriate lower order ANOVAs and Newman–Keuls multiple comparisons. In addition, an analysis of covariance was performed to factor out the influence of IF on DA levels.

To test for significant correlations other than effect of hormone treatment, a partial correlation analysis for behavior and extracellular DA levels, controlling for hormone treatment, was also performed. These partial correlations show the relationship between the two dependent variables (DA and behavior) after the effects of the independent variable (hormone) are factored out statistically. The following DA variables were investigated: average baseline level (BL); the maximum amount present in the estrous-female condition (E MAX); the maximum amount present in copulation sampling (C MAX); the maximum amount of increase in DA from baseline to estrous-female condition (E MAX INC); the maximum amount of increase in DA from baseline to copulation testing (C MAX INC); and the increase from average estrous-female condition to average copulation condition (E to COP).

Results

The two major metabolites of T were differentially effective in maintaining both copulation and extracellular DA in the MPOA. Stimulation of only androgen receptors (DHTB) or neither receptor type (oil) maintained almost no copulation (see Table 1). One oil-treated animal mounted, and one DHTB-treated animal mounted and intromitted, whereas none of the other oil ($n = 4$) or DHTB ($n = 5$) animals displayed any copulatory behavior. All EB-treated animals ($n = 7$) mounted and intromitted, but none ejaculated. Three of the EB + DHTB-treated animals ($n = 5$) copulated to ejaculation, and two only mounted and intromitted. All TP-treated animals ($n = 4$) ejaculated. Kruskal–Wallis tests indicated significant effects of hormone condition on mount frequency [$X^2(4) = 16.5$, $P < 0.002$], intromission frequency [$X^2(4) = 17.7$, $P < 0.001$], and ejaculation frequency [$X^2(4) = 18.5$, $P < 0.001$]. X^2 tests showed significant differences among groups on the percentages of animals exhibiting mounts ($X^2 = 17.3$, $P < 0.002$), intromissions ($X^2 = 21.324$, $P < 0.001$), and ejaculations ($X^2 = 19.1$, $P < 0.001$). Because DHT and oil groups had at most one animal that mounted or intromitted, and only TP and EB + DHTB groups ejaculated, ANOVAs or *t* tests for latency measures and IF_1 were calculated only for the groups that exhibited the relevant behavior. There were no significant differences in mount or intromission latencies among TP, EB, and EB + DHTB groups, or in IF_1, ejaculation latency, or postejaculatory interval between TP and EB + DHTB groups.

Post hoc multiple comparisons showed that the TP-treated group had more ejaculations than did EB- and DHTB-treated groups ($P = 0.05$ for each). (See Table 1 for all values.) The EB-treated group had more mounts and intromissions, compared to the oil-treated group, more mounts than the TP-treated group ($P = 0.05$ for each), and

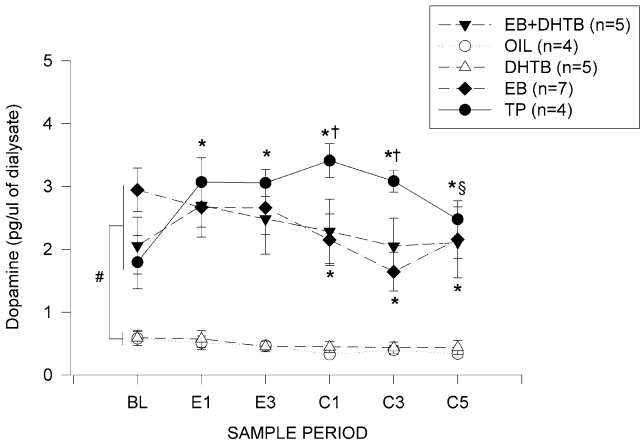

Fig. 1. Temporal changes in dialysate concentrations of dopamine in the medial preoptic area of male rat castrates during copulation testing. Each data point represents the mean ± SEM for 6-min samples collected during baseline (BL), two precopulatory periods with an estrous female behind a barrier (EST1 and EST3), and three periods after the barrier was removed and the animals were free to copulate (COP1, COP3, and COP5). (Although all samples were analyzed statistically, only the odd-numbered samples are depicted, for greater clarity.) *Significantly ($P < 0.05$) different from respective baseline (within-group); §Significantly ($P < .05$) different from respective COP 3 (within-group); †Significantly ($P < .05$) different from EB and E+DHTB groups (between-group); $^{\#}$Significant ($P < .05$) difference between Oil and DHTB groups and the three other groups for every sample period (between-group).

more intromissions than the DHTB-treated group ($P = 0.05$).

As with copulation, the DHTB- and oil-treated animals had the lowest basal DA levels and no female-stimulated DA increase (see Fig. 1). EB-treated animals had high basal DA levels, but showed no increase before or during copulation. Combined stimulation of estrogen and androgen receptors (TP and EB + DHTB groups) resulted in normal basal DA levels and a female-stimulated increase. A two-way mixed ANOVA detected significant effects of hormone treatment [$F(4, 20) = 16.10$, $P < 0.0001$], sample period [$F(9, 180) = 4.23$, $P < 0.0001$], and interaction [$F(36, 180) = 3.15$, $P < 0.001$] on extracellular DA (expressed in picograms per microliter of dialysate) (see Fig. 1). Because some groups had more intromissions, and therefore more sensory input, than other groups, we ran a comparable analysis using IF as a covariant. This analysis resulted in relatively little change, compared to ANOVA values, for treatment [$F(4, 19) = 10.50$, $P < 0.0001$], sample period [$F(9, 171) = 4.39$, $P < 0.0001$], or interaction [$F(36, 171) = 3.40$, $P < 0.0001$]. Therefore, we did not include IF as a covariant in further analyses.

One-way repeated-measure ANOVAs on each treatment group detected significant effects of sample period in TP-[$F(9, 180) = 6.61$, $P < 0.001$], EB-[$F(9, 180) = 8.51$, $P < .001$], and EB + DHTB-[$F(9, 180) = 2.28$, $P < .05$] treated animals. Newman–Keuls multiple comparison tests on TP-treated animals revealed a significant increase ($P < .05$) from average baseline (BL) to all estrous-female (EST) and copulation (COP) samples. In addition, there was a significant decrease from the last estrous-female (EST 4) and first copulation (COP 1) samples to the last copulation (COP 5) sample. In EB-treated animals there was a significant decrease from BL to the five COP samples. Although the one-way ANOVA was significant for EB + DHTB-treated animals, and a trend ($0.05 < P < 0.10$) toward an increase during EST periods was observed, no pairwise comparison was significant.

One-way ANOVAs for each sample period detected significant differences in extracellular DA levels among the hormone treatment groups in all sample periods: BL, [$F(4, 20) = 9.52$, $P < 0.001$]; EST 1, [$F(4, 20) = 12.76$, $P < 0.001$]; EST 2, [$F(4, 20) = 14.23$, $P < 0.001$]; EST 3, $F[4, 20) = 11.19$, $P < 0.001$]; EST 4, [$F(4, 20) = 14.22$, $P < 0.001$]; COP 1, [$F(4, 20) = 13.05$, $P < .001$]; COP 2; [$F(4, 20) = 23.99$, $P < .001$]; COP 3, [$F(4, 20) = 15.01$, $P < .001$]; COP 4, [$F(4, 20) = 12.98$, $P < .001$]; and COP 5, [$F(4, 20) = 8.07$, $P < 0.001$]. Newman–Keuls multiple-comparison tests ($P < 0.05$) revealed that oil- and DHTB-treated animals had significantly lower extracellular DA levels than other groups at all sampling periods. Additionally, TP-treated animals had higher DA levels than EB-treated animals during the first four COP periods and higher DA levels than EB + DHTB-treated animals during the first three COP sampling periods.

Partial correlation analysis, controlling for hormone treatment, detected significant relationships between extracellular DA and some of the observed copulatory behaviors. A greater number of intromissions (IF) was correlated with an increase from the average estrous-female sample to the average copulation sample (E to COP, pr = 0.59). A greater number of ejaculations (EF) was negatively correlated with higher baseline levels of extracellular DA (BL, pr = −.58) but positively correlated with maximum increase of extracellular DA from baseline to highest estrous-female sample (E MAX INC, pr = 0.66), an increase from average estrous-female sample to the average copulation sample (E to COP, pr = 0.50), and with baseline to the highest copulation sample (C MAX INC, pr = 0.74).

Discussion

There has been considerable variability, both between and within studies, in the ability of E, either alone or in combination with DHT, to restore copulation in castrates (Davidson, 1969; Baum and Vreeburg, 1973; Larsson et al., 1973; Feder et al., 1974; Lodder and Baum, 1977; Hawkins et al., 1988; Vagell and McGinnis, 1998; reviewed in Hull et al., 2002). Although the doses that were used in the present experiment provided supraphysiological levels of hormones, we chose doses in the ranges that were previously most effective in restoring copulation in order to maximize the probability of observing at least some copulatory behavior. It is possible that these doses may have

resulted in nongenomic effects. However, such nongenomic effects would be expected to enhance, rather than impair, copulation (Cross and Roselli, 1999). Thus, it seems unlikely that the lack of ejaculatory ability in our EB-treated animals resulted from the supraphysiological levels of the hormone acting on membrane-bound estrogen receptors.

One reason for the impairment of ejaculatory ability in the EB group may be the lack of androgenic stimulation of the genitals (Baumgardner and Dewsbury, 1980; Sachs, 1983) and the spinal neurons that control genital reflexes (Hart, 1973). However, the combination of E and DHT was more effective in stimulating copulation, even in males whose pudendal nerve had been sectioned, thereby removing the major source of sensory input from the penis; this suggests that E and DHT act synergistically in the brain (Lodder and Baum, 1977). Furthermore, at least some E-treated castrates have been able to ejaculate, apparently as a result of a "behavioral cascade" organized in the brain (Sachs, 1983), despite the lack of *ex copula* reflexes (Meisel et al., 1984). It is possible that some E-treated males in the present study may have been able to ejaculate if the behavioral tests had been extended for a longer time.

A consistent relationship between female-stimulated MPOA DA release and the ability to copulate was previously observed. Both the postcastration decline in copulation (Hull et al., 1995) and its restoration with exogenous T (Putnam et al., 2001) were associated with the loss and restoration, respectively, of the MPOA DA response to a female. Basal extracellular DA was also significantly lower in oil-treated castrates, compared to intact males or TP-treated castrates; however, DA synthesis and storage in the MPOA were at least normal, and perhaps enhanced, in the castrates (Du et al., 1998). Thus, castrates could synthesize and store DA, but showed deficits in its release. Furthermore, castration did not affect immunoreactivity for tyrosine hydroxylase, the rate-limiting enzyme for DA synthesis, but did decrease nitric oxide synthase, which produces nitric oxide (NO) (Du and Hull, 1999). We have previously shown that DA release in both basal (Lorrain and Hull, 1993) and female-stimulated (Lorrain et al., 1996) conditions is regulated by NO. Therefore, the low extracellular levels of MPOA DA in castrated male rats appear not to be due to a deficit in DA synthesis, but may be related to a deficit in NO-mediated release.

The suboptimal copulatory ability of EB-treated males in this study suggests that normal basal DA levels can maintain some copulation, but that the increase immediately before and during copulation is important for facilitation of ejaculation. Furthermore, although DHTB was ineffective by itself, addition of DHTB to EB enabled at least some DA response to the female and ejaculatory ability in the majority of animals. The female-stimulated MPOA DA response may be elicited by the medial amygdala (MeA). Male rats with MeA lesions had normal basal extracellular DA levels in the MPOA, but no female-stimulated increase (Dominguez et al., 2001). These animals had fewer than half the number of ejaculations of the sham-lesion controls. Thus, as in the EB-treated castrates in the present experiment, normal basal levels of MPOA DA were sufficient for suboptimal copulation; however, an additional DA increase was required to increase the number of ejaculations. Large excitotoxic lesions of the amygdala almost eliminated copulation, but microinjections of the DA agonist apomorphine into the MPOA restored copulatory ability (Dominguez et al., 2001). Therefore, a major way in which the MeA promotes male sexual behavior may be by increasing DA release in the MPOA shortly before and during copulation. Furthermore, the MeA is one site at which androgens act to facilitate copulation. DHT implants into the MeA, combined with subthreshold systemic injections of E, were sufficient to activate behavior in castrated male rats (Baum et al., 1982). However, DHT implants without systemic E were ineffective in male hamsters (Wood, 1996).

The significant correlations between DA measures and specific behavioral parameters, after controlling statistically for the effects of hormone treatment, suggest that extracellular DA made independent contributions to copulatory behavior. Thus, hormones are permissive for DA to exert its own background and moment-by-moment effects. An increase in extracellular DA from baseline level in response to an estrous female (i.e., E MAX INC) and a large increase in DA levels from baseline to copulation samples were associated with copulatory measures. A further increase in extracellular DA from the time of exposure to an estrous female to the copulation sample (i.e., E to COP) emerged most frequently as an important predictor of copulatory behavior. The negative association between baseline DA levels and number of ejaculations may best be explained by the copulatory behavior of the EB group. Although these animals had high basal levels of DA and numerous intromissions, none ejaculated. Perhaps high levels of extracellular DA in basal conditions down-regulate DA receptors, so that a physiologically significant DA increase is necessary to stimulate ejaculation.

The suggestion that normal basal DA levels are sufficient for suboptimal copulation, but that increased DA release facilitates ejaculation is consistent with previous data from our lab. Low doses of the classic DA agonist apomorphine, microinjected into the MPOA, facilitated *ex copula* erections via D_1-like receptors, whereas higher doses facilitated seminal emissions via D_2-like receptors (Hull et al., 1992). Thus, small increases above basal levels may stimulate parasympathetically mediated erection via D_1-like receptors, whereas higher increases shift autonomic balance to favor sympathetically mediated ejaculation via D_2-like receptors.

The correlations between MPOA DA release and copulatory behavior do not, themselves, imply causation. It is possible that the increased DA release was caused by the behavior rather than vice versa. However, DA levels increased during exposure to an estrous female, before copulation began. Furthermore, factoring out the effects of sen-

sory input from intromissions did not affect *F* values in analyses of extracellular DA levels. Finally, previous microinjection studies have shown that the DA agonist apomorphine, microinjected into the MPOA, increased the rate and efficiency of copulation (Hull et al., 1986) and increased *ex copula* reflexes (Pehek et al., 1989; Hull et al., 1992). Apomorphine in the MPOA also partially restored copulation in long-term castrates that had not mounted for at least a month (Scaletta and Hull, 1990) and completely restored it in males with large excitotoxic lesions of the medial amygdala (Dominguez and Hull, 2001). Conversely, microinjections of DA antagonists impaired sexual motivation, copulation, and genital reflexes (Pfaus and Phillips, 1989; Warner et al., 1991). Thus, MPOA DA influences sexual behavior. Finally, male mice lacking the classic estrogen receptor (ERα) showed little copulation when injected with vehicle, but exhibited full copulatory behavior when injected systemically with apomorphine (Wersinger and Rissman, 2000). Therefore, a major function of E may be to increase DA release.

Hormonal regulation of DA release is at least somewhat site-specific. Hormonal manipulations affected extracellular DA in the striatum of female, but not male, rats (reviewed in Becker, 1999). There are apparently contradictory reports of the effects of castration on DA levels in the nucleus accumbens. Castration in adulthood decreased DA in nucleus accumbens tissue punches (Mitchell and Stewart, 1989). However, amphetamine released more DA in dialysate from the nucleus accumbens of prepubertally castrated males, suggesting that there was more DA stored in tissue (Hernandez et al., 1994). Dopaminergic regulation of copulation is also somewhat site-specific; microinjection cannulae or microdialysis probes located in areas adjacent to the MPOA were not associated with behavioral or neurotransmitter changes, respectively (Hull et al., 1986, 1995). However, DA is also released in the paraventricular nucleus (Melis et al., 2003) and in the nucleus accumbens (Damsma et al., 1992) before and during copulation and in the dorsal striatum only during copulation (Damsma et al., 1992) in male rats. MPOA DA release also shows at least some behavioral specificity. Eating a palatable food (Hull et al., 1993), voluntary exercise in a running wheel, or exposure to a male rather than a female (Hull et al., 1995) elicited a nonsignificant increase in MPOA DA activity.

In summary, the close relationship between extracellular MPOA DA levels and the ability of male rats to copulate (Hull et al., 1995; Putnam et al., 2001) was confirmed in the present study. The ability to achieve a more robust increase in extracellular DA before and during copulation resulted in a general facilitation of copulation. Furthermore, the presence of EB, whether alone, in combination with DHTB, or aromatized from TP, increased extracellular DA in basal conditions. EB-alone also promoted suboptimal copulation. However, the additional presence of androgen (TP or EB + DHTB) was necessary for female-stimulated DA release and for ejaculation. Androgen alone (DHTB) was ineffective in maintaining copulation or basal or female-stimulated DA release. Thus, the two major metabolites of T play complementary roles in the maintenance of copulation and of DA release in the MPOA.

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