The medial preoptic area (MPOA) is an important integrative site for male sexual behavior. We have reported an increase in dopamine (DA) release in the MPOA of male rats shortly before and during copulation. Postcastration loss of copulatory ability mirrored the loss of the precopulatory DA response to an estrous female. The present study investigated the time courses of restoration, rather than loss, of the MPOA DA response to a receptive female and of copulation in long-term castrates. Male rats were castrated and tested for loss of copulatory ability 21 days later. They then received 2, 5, or 10 daily subcutaneous injections of testosterone propionate (TP, 500 µg) or oil. Microdialysate samples were collected from the MPOA during baseline, exposure to a female behind a barrier, and copulation. Extracellular DA was measured using HPLC–EC. None of the six 2-day-TP-treated animals copulated, nor did they show elevated DA release in the MPOA in the presence of a receptive female. Five of the nine 5-day-TP-treated animals ejaculated; three intromitted without ejaculating; and one failed to copulate, with all but the noncopulating animal showing elevated DA release. All of the six 10-day-TP-treated animals copulated and also demonstrated an increase in MPOA DA. None of the oil controls copulated or showed an increase in DA release. Therefore, a consistent relationship between MPOA DA release during exposure to a receptive female and the subsequent ability of the male to copulate was observed.

Key Words: dopamine; medial preoptic area; testosterone; copulation; microdialysis.
Moses, and Hull, 1994). Mas and his colleagues reported increased levels of DA and its major metabolites in homogenates from the POA, obtained immediately after the first intromission or the first ejaculation (Mas, Rodriguez del Castillo, Guerra, Davidson, and Battaner, 1987). Furthermore, levels of DA metabolites in the MPOA (Hull, Eaton, Moses, and Lorrain, 1993) and, slightly more lateral, in the POA (Fumero, Fernandez-Vera, Gonzalez-Mora, and Mas, 1994; Mas, Fumero, and Gonzales-Mora, 1995) were increased during copulation. Preoptic DA levels were too low to be measured in those early studies; therefore, only metabolite levels were reported.

Subsequent studies, using more sensitive equipment, were able to detect DA in the MPOA. We observed a consistent relationship between MPOA DA release during a precopulatory period, during which a male was exposed to a receptive female behind a barrier, and the subsequent ability of the male to copulate after the barrier was removed (Hull, Du, Lorrain, and Matuszewich, 1995). There appears to be substantial behavioral specificity to the MPOA DA response, since running on an activity wheel, eating a highly palatable food, or the presence of another male all failed to produce a similar increase in DA release (Hull et al., 1993, 1995).

Repeated systemic or intracranial administration of testosterone propionate (TP) has proven to be effective in both the maintenance and restoration of sexual activity in male rat castrates (Beach and Holtz-Tucker, 1949; Davidson, 1966a,b). Recently administered testosterone is also a permissive factor for the MPOA DA response to an estrous female (Hull et al., 1995). In that study, sexually experienced rats were castrated and injected daily for 1 or 2 weeks with either 200 µg TP or oil vehicle. All of the TP-treated animals showed the precopulatory dopamine response, and all of them copulated after the barrier was removed. The oil-treated castrates that showed the precopulatory dopamine response subsequently copulated, while those that failed to show the anticipatory dopamine response failed to copulate (Hull et al., 1995).

The reemergence of sexual activity in animals whose copulatory ability has been extinguished does not occur for a number of days after treatment begins, depending on the dose of testosterone used (Davidson, 1966b). Thus, restoration of copulation in long-term castrates typically requires at least 5–10 days of testosterone administration, even though testosterone has been shown to increase neural firing in the MPOA within minutes (Pfaff and Pfaffman, 1969) or seconds (Yamada, 1979).

The present study used in vivo microdialysis and high-performance liquid chromatography with electrochemical detection (HPLC–EC) to investigate the time courses of restoration of the MPOA DA response to a receptive female and of copulation in long-term male rat castrates.

**MATERIALS AND METHODS**

**Animals**

Adult male Long–Evans Blue–Spruce rats (250–300 g), purchased from Harlan Sprague-Dawley (Indianapolis, IN), were individually housed in a temperature- and humidity-controlled environment with food and water available ad libitum. They were maintained on a 14:10 reverse light cycle, with lights off at 11:00 h, and weighed daily to monitor health status and to accustom them to handling procedures.

All animals were screened for copulatory ability 1 week after arrival, using ovariectomized females of the same strain. The females were brought into estrus with injections of estradiol benzoate (EB, 20 µg sc) (Sigma, St. Louis, MO), administered 48 h, and progesterone (P) (500 µg sc) (Sigma) 4 h prior to testing. The sexual receptivity of each female was verified by permitting three intromissions by a stud male in his home cage. Experimental males were considered sexually active after achieving two ejaculations.

**Surgery**

**Orchidectomy.** All sexually active male rats were anesthetized with an injection (im) of ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4 mg/kg) and were 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 signs of infection. All procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the local IACUC.

**Intracranial surgery and cannula implantation.** One week after orchidectomy, experimental animals were anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4 mg/kg) im, prepared for surgery, and placed in a Kopf stereotaxic
frame with the incisor bar set 5 mm above the interaural line. All rats were implanted with a 15-mm, 23-gauge thin-wall stainless steel guide cannula, aimed at and ending 2 mm above the left MPOA (mm from bregma: AP, +2.2; ML, +0.2; DV, −6.2; Pellegrino et al., 1979). (See Fig. 1 for the intended location of probes.) The skull was exposed and a small hole was drilled above the MPOA. Prior to lowering the guide cannula, three additional holes (one in each of the remaining intact bones surrounding bregma) were drilled and implanted with screws to provide anchor-age for the cannula assembly. The guide cannula was lowered to the appropriate depth and dental cement was spread around the screws and guide cannula. 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-gauge stainless steel tubing and cut to the length of the guide cannula was inserted to prevent the entry of foreign material. (Details of cannula construction are described in Hull et al., 1986).

Hormone Treatment

Animals were tested to confirm loss of copulatory ability 21 days after castration and then were randomly assigned to a hormone treatment group or a control (oil) group. Thus, each animal received 2, 5, or 10 daily subcutaneous injections of either 500 µg of testosterone propionate (TP) (Sigma) in 0.1 ml olive oil or 0.1 ml of olive oil as a control.

Microdialysis

Microdialysis probes using a concentric flow design were used for all dialysis collection. A 3-mm length of dialysis membrane [MW cutoff 12,000, 210 µm outside diameter (o.d.), Spectra-Por] was glued to one end of a 27-gauge 15-mm stainless steel shaft with waterproof epoxy. The end was plugged and 2 mm inactivated with epoxy to maintain an active dialyzing length of 1 mm. A 20-cm length of polyethylene (PE) 20 tubing was fitted to the opposite end of the shaft to serve as the inlet for the perfusion medium. A 20-cm length of silica capillary tubing [125 µm o.d., 50 µm inner diameter (i.d); Polymicro Technologies, Phoenix, AZ] was inserted into the dialysis tube, providing an outlet for the perfusion medium. Samples were collected at 6-min intervals into 250-µl centrifuge tubes and either injected into the HPLC system immediately or frozen in dry ice, followed by storage in a −80°C supercool freezer. The dialysis perfusion medium was a modified Ringer’s solution (in mM: 138 NaCl, 2.7 KCl, and 1.2 CaCl₂, pH 7.0). Flow was controlled by a Harvard Model 22 syringe infusion pump.

On the day of testing, subjects were lightly anesthe-tized 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 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 with a wire bottom that was suspended over the male’s home cage, permitting visual, auditory, and olfactory stimulation, but not direct physical interaction. An obturator made from 27-gauge stainless steel tubing and cut to the length of the guide cannula was inserted to prevent the entry of foreign material. (Details of cannula construction are described in Hull et al., 1986.)
HPLC–EC

DA was assayed using capillary HPLC–EC. Samples were loaded via a Rheodyne injector valve, which delivered a 500-nl volume to an LC-Packings Fusica C18 capillary column. The mobile phase, consisting of 30 mM citric acid, 50 mM sodium acetate, 0.027 mM Na$_2$EDTA, 0.25 mM octyl sodium sulfate, and 2.5% acetonitrile (degassed and pH 3.8), was delivered by a Gilson Model 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 an Ag/AgCl reference electrode. The amount of DA present in dialysate samples was evaluated using a Gilson Unipoint program.

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 (or the first intromission if not preceded by a 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; intromission frequency (IF), number of mounts with penile insertion; ejaculation frequency (EF), number of ejaculations per 30-min test. Animals that did not exhibit a behavior were assigned 1800 s (the length of the test) as the latency for each behavior.

Histology

Immediately after copulation testing and final dialysate collection, subjects were deeply anesthetized with sodium pentobarbital (ip), dye was infused through their probes, and the animals were decapitated. Brains were removed and coronal sections cut (40 μm) on a freezing microtome. Slices were mounted on slides, and probe placement was histologically verified at that time. The slices were then dehydrated, stained with cresyl violet, and coverslipped with Permount.

Data Analyses

Data from 40 animals that had less than 10% variation among baseline samples, had correct probe placements, and from which dialysate samples were successfully obtained were included in statistical analyses. Copulatory behaviors were first compared among hormone treatment groups with a two-way analysis of variance (ANOVA), followed by one-way ANOVAs and Newman–Keuls multiple comparisons. Dopamine contents in dialysate (expressed as a percentage of the mean of the last three baseline peak heights) were compared across the final baseline, estrous female exposure, and copulation intervals using a three-way repeated measures ANOVA (hormone × days of treatment × sample period as a repeated factor). All significant effects were further analyzed with appropriate lower order ANOVAs and Newman–Keuls multiple comparisons. The Greenhouse–Geisser correction procedure was used when the sphericity assumption was violated. To examine significant correlations other than the effects of treatment, a partial correlation analysis for behavior and extracellular DA levels, controlling for hormone (testosterone vs oil) and number of days of treatment, was performed. These partial correlations show the relationship between the two dependent variables (DA and behavior) after the effects of the independent variables (hormone and days) are factored out statistically.

RESULTS

None of the six 2-day-TP-treated animals copulated. On the other hand, five of the nine 5-day-TP-treated animals copulated to ejaculation; three intromitted but did not ejaculate; and one failed to copulate. All of the six 10-day-TP-treated animals ejaculated. None of the six 2-day, eight 5-day, or five 10-day oil controls copulated.

Two-way ANOVAs showed significant main effects of hormone on all behavioral measures and of days of treatment on all behavioral measures except mount latency. There were also significant interactions for all behavioral measures. One-way ANOVAs for testosterone-treated animals revealed significant effects of days of treatment on all observed behavioral parameters except mount latency: mount frequency (MF) \[F(2, 34) = 12.76, P < 0.001\], intromission latency (IL) \[F(2, 34) = 65.12, P < 0.001\], intromission frequency (IF) \[F(2, 34) = 10.51, P = 0.001\], ejaculation latency (EL) \[F(2, 34) = 10.27, P < 0.001\],
ejaculation frequency (EF) \[F(2, 34) = 15.92, P < 0.001\], and postejaculatory interval (PEI) \[F(2, 34) = 13.97, P < 0.001\]. (See Table 1 for group means.) Posthoc multiple comparisons among the TP-treated groups showed that IL, EL, and PEI were significantly shorter in the 5- and 10-day-TP-treated groups than in 2-day-TP-treated males \((P < 0.01)\). MF was significantly greater in the 10-day-TP-treated group than in the 2- and 5-day groups \((P < 0.01)\), and the 5-day animals had more mounts than the 2-day group \((P < 0.05)\). IF and EF were significantly greater in 5- and 10-day-TP-treated animals than in the 2-day group \((P < 0.01)\), and EF was also greater in the 10-day than in the 5-day animals \((P < 0.05)\). There were no significant differences among the oil-treated groups on any measure.

A three-way repeated measures ANOVA on DA levels revealed significant main effects of hormone, days of treatment, and sample period and significant two- and three-way interactions among all of those factors: hormone × days × sample: \(F(10, 122) = 2.18, P < 0.04\); hormone × days: \(F(2, 122) = 6.21, P < 0.005\); sample × days: \(F(5, 122) = 2.29, P < 0.03\); sample × hormone: \(F(5, 122) = 8.15, P < 0.0001\); sample: \(F(5, 122) = 2.54, P < 0.05\); hormone: \(F(1, 122) = 30.77, P < 0.0001\); days: \(F(2, 122) = 8.16, P < 0.001\). (See Fig. 2.) Posthoc analysis of oil-treated animals revealed main effects only of sample period \([F(5, 122) = 2.78, P < 0.05]\), where DA levels in the third (C3) and fifth (C5) copulation samples were significantly lower than baseline \((P < 0.05)\). On the other hand, the two-way ANOVA on TP-treated animals revealed a significant interaction [sample period × days: \(F(10, 122) = 4.83, P < 0.001\)] and main effects of sample period \([F(5, 122) = 7.67, P < 0.001]\) and days \([F(2, 122) = 40.30, P < 0.001]\). One-way ANOVAs and posthoc comparisons revealed that there was no effect of sample period on DA levels in 2-day-TP-treated animals \([F(5, 122) = 1.07, \text{ns}]\). For 5-day-TP-treated animals, there was a significant effect of sample period on DA levels \([F(5, 122) = 2.56, P < 0.05]\), where the DA levels in the third copulation sample (C3) were significantly higher than those in baseline \((P < 0.05)\). For 10-day-TP-treated animals, there was a significant effect of sample period on DA levels \([F(5, 122) = 7.76, P < 0.001]\). The DA levels in the third copulation sample were significantly higher than those in baseline, first and third estrous female (E1 and

### Table 1

<table>
<thead>
<tr>
<th>Hormone condition</th>
<th>ML (SE)</th>
<th>IL (SE)</th>
<th>EL (SE)</th>
<th>PEI (SE)</th>
<th>MF (SE)</th>
<th>IF (SE)</th>
<th>EF (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Day TP n = 6</td>
<td>941.2 ± 377.3</td>
<td>1800.0 ± 0.0</td>
<td>1800.0 ± 0.0</td>
<td>1800.0 ± 0.0</td>
<td>1.8 ± 1.3</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>5-Day TP n = 9</td>
<td>212.2 ± 198.6</td>
<td>351.1 ± 189.1</td>
<td>1099.7 ± 231.3</td>
<td>1129.9 ± 212.8</td>
<td>5.8 ± 1.4</td>
<td>10.1 ± 3.0</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>10-Day TP n = 6</td>
<td>40.7 ± 16.3</td>
<td>177.7 ± 33.4</td>
<td>988.3 ± 73.8</td>
<td>880.7 ± 59.9</td>
<td>12.0 ± 1.3</td>
<td>9.8 ± 0.9</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>Mean TP n = 21</td>
<td>373.3 ± 154.3</td>
<td>715.8 ± 172.6</td>
<td>1268.2 ± 123.5</td>
<td>1250.4 ± 105.4</td>
<td>6.43 ± 1.1</td>
<td>7.1 ± 1.6</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>2-Day oil n = 6</td>
<td>937.2 ± 378.7</td>
<td>1800.0 ± 0.0</td>
<td>1800.0 ± 0.0</td>
<td>1800.0 ± 0.0</td>
<td>1.8 ± 1.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>5-Day oil n = 8</td>
<td>1171.9 ± 311.0</td>
<td>1800.0 ± 0.0</td>
<td>1800.0 ± 0.0</td>
<td>1800.0 ± 0.0</td>
<td>2.9 ± 1.7</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>10-Day oil n = 5</td>
<td>1800.0 ± 0.0</td>
<td>1800.0 ± 0.0</td>
<td>1800.0 ± 0.0</td>
<td>1800.0 ± 0.0</td>
<td>0.0 ± 1.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Mean oil n = 19</td>
<td>1263.3 ± 185.1</td>
<td>1800.0 ± 0.0</td>
<td>1800.0 ± 0.0</td>
<td>1800.0 ± 0.0</td>
<td>1.8 ± 0.8</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>

Note. Results are means ±SE for behavioral parameters during a 30-min copulation test. ML, mount latency (seconds); MF, number of mounts; IL, intromission latency (seconds); IF, number of intromissions; EL, ejaculation latency (seconds); EF, number of ejaculations; PEI, time between ejaculation and the next intromission (seconds); TP, testosterone propionate. Posthoc comparisons following one-way ANOVAs for TP-treated animals \((P < 0.05)\):

- `a` Significantly different from 2-day TP group.
- `b` Significantly different from 5-day TP group.
- `c` Significantly different from mean of oil-treated groups. There were no significant differences due to days of treatment for oil-treated animals.
E3), and fifth copulation (C5) samples ($P < 0.01$). In addition, the DA levels in the first copulation sample (C1) were higher than those in baseline ($P < 0.01$), third estrous female (E3, $P < 0.05$), and fifth copulation (C5, $P < 0.05$) samples.

Partial correlation analysis, controlling for both hormone condition and number of days of treatment, detected significant relationships between extracellular DA and some of the observed copulatory behaviors. (See Table 2 for all values.) A large increase in DA was associated with shorter mount, intromission, and ejaculation latencies and postejaculatory intervals, as seen in the negative correlations between these measures and various sample periods. ML was negatively correlated with DA increases in the first estrous female sample and the first two copulation samples. IL was negatively correlated with DA increases in all of the estrous female and copulation samples. Both EL and PEI were negatively correlated with DA increases in both estrous female samples. Furthermore, an increase in MPOA DA was associated with a greater number of mounts, intromissions, and ejaculations. MF was positively correlated with DA in the first and third copulation samples, whereas IF was positively correlated with all estrous female and copulation samples. EF was positively correlated only with the first estrous female sampling period. Thus, a large increase in DA upon exposure to an estrous female was associated with a shorter latency to ejaculate, a shorter interval before resuming sexual activity, and a greater number of ejaculations.

DISCUSSION

The results of the present study concur with those in a previous report of a consistent relationship between MPOA dopamine release during exposure to an estrous female behind a barrier and the subsequent ability of a male to copulate (Hull et al., 1995). In that previous study, sexually experienced rats were castrated and injected daily for 1 or 2 weeks with either 200 μg TP or oil vehicle. All of the TP-treated animals showed the precopulatory dopamine response, and all of them copulated after the barrier was removed. The oil-treated castrates that showed the precopulatory

TABLE 2

<table>
<thead>
<tr>
<th>Sample Period</th>
<th>ML</th>
<th>IL</th>
<th>EL</th>
<th>PEI</th>
<th>MF</th>
<th>IF</th>
<th>EF</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>-0.37*</td>
<td>-0.60*</td>
<td>-0.53*</td>
<td>-0.57*</td>
<td>0.19</td>
<td>0.34*</td>
<td>0.43*</td>
</tr>
<tr>
<td>E3</td>
<td>-0.13</td>
<td>-0.45*</td>
<td>-0.37*</td>
<td>-0.40*</td>
<td>0.05</td>
<td>0.56*</td>
<td>0.25</td>
</tr>
<tr>
<td>C1</td>
<td>-0.39*</td>
<td>-0.50*</td>
<td>-0.24</td>
<td>-0.26</td>
<td>0.41*</td>
<td>0.63*</td>
<td>0.17</td>
</tr>
<tr>
<td>C3</td>
<td>-0.36*</td>
<td>-0.50*</td>
<td>-0.27</td>
<td>-0.27</td>
<td>0.38*</td>
<td>0.51*</td>
<td>0.26</td>
</tr>
<tr>
<td>C5</td>
<td>-0.21</td>
<td>-0.39*</td>
<td>-0.09</td>
<td>-0.13</td>
<td>0.30</td>
<td>0.58*</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Note. Partial correlation coefficients, controlling for hormone treatment and days of treatment, indicating the relationship between behavioral measures and an increase in DA from baseline in dialysate collected from the MPOA during exposure to an estrous female (E1 and E3) and during copulation (C1, C3, and C5) are shown. Behavioral measures: ML, latency to mount; MF, total number of mounts in a 30-min test; IL, latency to intromit; IF, number of intromissions in a 30-min test; EL, latency to ejaculate; EF, total number of ejaculations in a 30-min test; PEI, time interval between ejaculation and the next intromission.

* Significance ($P < 0.05$): departure from zero.
dopamine response subsequently copulated, while those that failed to show the anticipatory dopamine response failed to copulate. The present study focused on restoration, rather than postcastration loss of copulation. In this experiment, an increase in the release of MPOA DA in response to an estrous female was once again predictive of an animal’s ability to copulate. Furthermore, it appears that 5 days is a threshold period for restoration of both the DA response and sexual behavior. Two days of systemic testosterone replacement did not restore copulation in male rat castrates, nor did it restore the MPOA DA release during exposure to a receptive female. Five days of testosterone replacement, however, restored both sexual behavior and the precopulatory MPOA DA response in most animals; all but one of the 5-day-TP-treated animals achieved intromissions, and over half copulated to ejaculation. All animals receiving 10 days of TP replacement copulated and showed the precopulatory MPOA DA release. None of the 2-, 5-, or 10-day oil controls copulated or showed a DA response to the female. Thus, in the present experiment, the restoration of copulatory ability by TP in long-term castrates mirrored the responsiveness of MPOA DA release in the presence of a receptive female.

The time course of restoration of copulation in long-term castrates observed in this experiment is consistent with earlier reports that the reemergence of sexual activity in such animals does not occur for 5 to 10 days after the initiation of systemic TP treatment (Beach and Holtz-Tucker, 1949; Davidson, 1966b; Goldfoot and Baum, 1971; McGinnis and Mirth, 1986; McGinnis, Mirth, Zebrowski, and Dreifuss, 1989). Indeed, in prepuberally castrated male rats that received daily testosterone injections beginning on day 36 of age, 5 days of treatment was required to induce copulatory behavior in most nonshocked animals (Goldfoot and Baum, 1972). Restoration of copulation was prevented by systemic administration of an antiandrogen (McGinnis and Mirth, 1986). On the other hand, testosterone increased neural firing in the MPOA within minutes (Pfaff and Pfaffman, 1969) or even seconds (Yamada, 1979). Therefore, longer term genomic effects are necessary for the behavioral restoration. This conclusion was supported by the finding that the protein synthesis inhibitor anisomycin, implanted into the MPOA, prevented the restoration of copulation by systemically administered T (McGinnis and Kahn, 1997).

Another important finding of the present study was that of significant correlations between increases in MPOA DA during the various sample periods and specific behavioral parameters, after controlling statistically for the effects of hormone treatment and duration of treatment. A DA increase in the first estrous female sample (E1) emerged most frequently as being associated with the observed behavioral parameters. Thus, the greater the increase in DA levels during the initial exposure to an estrous female, the greater the number of intromissions and ejaculations, and the shorter the latency to mount, intromit, and ejaculate, as well as to resume copulation after an ejaculation. The ability to maintain an increase in MPOA DA release through the third estrous female sample period (E3) was associated with a greater number of intromissions throughout the test period and shorter latencies to intromit, ejaculate, and resume copulation after ejaculation. The higher the DA increases during the early copulation samples (C1 and C3), the more mounts and intromissions the animal displayed and the shorter were the latencies to mount and intromit. Interestingly, it was only the amount of initial increase of MPOA DA in the presence of an estrous female that was associated with the ability to ejaculate more frequently, and only the DA increases in the two estrous female samples were correlated with more rapid ejaculation.

The fact that precopulatory DA levels provided more consistent correlations with copulation measures than did DA levels during copulation may imply that high precopulatory DA initiates ongoing neural processes that facilitate subsequent behavior. There is evidence for a facilitative effect on copulation of preexposure to an estrous female (de Jonge, Oldenburger, Louwerse, and Van De Poll, 1992). Therefore, such exposure appears to initiate ongoing processes that increase the subsequent copulatory ability of the male. However, it is possible that precopulatory DA increases also reflect the immediate responsiveness of DA release mechanisms to sexually relevant stimuli. Thus, the initial exposure to the female may elicit the greatest change in DA release, but there may also be fluctuations in DA release during copulation that are too small or too brief to be detected with microdialysis.

One reason that increases in DA levels during the copulation samples provided somewhat fewer correlations with copulatory measures may have been because DA levels decrease after an ejaculation. Thus, although mean DA levels were slightly (not significantly) higher during C1 and C3 than during E1, for the 10-day-TP-treated animals, there was more variability in the copulation samples, perhaps related to the timing of ejaculation. Nevertheless, the numerous significant correlations between MPOA DA increases
and copulation measures support the hypothesis that the MPOA DA response is an important factor regulating copulatory behavior. Although correlations do not imply causation, we have previously reported that microinjections of DA agonists into the MPOA facilitate copulation (Hull et al., 1986) and genital reflexes (Pehek, Thompson, and Hull, 1989), whereas DA antagonists impair copulation, genital reflexes, and sexual motivation (Warner, Thompson, Markowski, Loucks, Bazzett, Eaton, and Hull, 1991).

Testosterone regulates not only the DA response to an estrous female, but also basal DA levels in the MPOA. Using the no-net-flux method, we have shown that basal extracellular DA concentrations in the MPOA were lower in castrates than in intact males (Du, Lorrain, and Hull, 1998). This general deficit in MPOA DA release in the absence of testosterone appeared not to be due to decreased DA synthesis. Analyses of tissue punches taken from the MPOA showed that castrates actually had more DA stored in tissue than did gonadally intact males (Du et al., 1998). Furthermore, when male rats were systemically injected with amphetamine, more DA was released in the castrated subjects than in the gonadally intact rats (Du et al., 1998). Amphetamine depletes vesicular stores of DA and reverses the membrane DA transporter (Sulzer, Chen, Lau, Kristensen, Rayport, and Ewing, 1995). These data suggest that castrates actually have more DA available for release than intact males, but may not be able to release it.

One factor that may be important for the regulation of DA release in the MPOA is nitric oxide (NO). NO has been reported to increase the release of DA from striatal slices (Hanbauer, Wink, Osawa, Edelman, and Gally, 1992; Zhu and Luo, 1992). In a previous study, striatal slices (Hanbauer, Wink, Osawa, Edelman, and Gally, 1992) has been reported to increase the release of DA from tissue punches taken from the MPOA, suggesting that castrates had more DA stored in tissue than did gonadally intact males (Du et al., 1998).

Furthermore, when male rats were systemically injected with amphetamine, more DA was released in the castrated subjects than in the gonadally intact rats (Du et al., 1998). Amphetamine depletes vesicular stores of DA and reverses the membrane DA transporter (Sulzer, Chen, Lau, Kristensen, Rayport, and Ewing, 1995). These data suggest that castrates actually have more DA available for release than intact males, but may not be able to release it.

One factor that may be important for the regulation of DA release in the MPOA is nitric oxide (NO). NO has been reported to increase the release of DA from striatal slices (Hanbauer, Wink, Osawa, Edelman, and Gally, 1992; Zhu and Luo, 1992). In a previous study, the administration of the NO precursor L-arginine through a microdialysis probe increased DA release in the MPOA of male rats (Lorrain and Hull, 1993). An inhibitor of NO synthase (NOS), the enzyme that converts L-arginine to L-citrulline, thus producing NO, blocked this increase. In a subsequent study, reverse dialysis of a NOS inhibitor also blocked the DA increase during copulation that was seen in males receiving the inactive isomer (Lorrain, Matuszewich, Howard, Du, and Hull, 1996). Furthermore, castration decreased, and testosterone restored, the number of NOS-immunoreactive neurons in the MPOA (Du and Hull, 1999). This effect appeared to be relatively site specific, since no differences were found between castrated and intact males in several other areas that were examined, including the paraventricular nucleus of the hypothalamus and the amygdala. These data suggest that NO may play an important, if not essential, role in the increase in MPOA DA release during copulation. Experiments are under way to determine whether testosterone-induced changes in NOS and in tissue (stored) DA follow the same 5-day pattern.

In summary, the consistent relationship between MPOA DA release during a precopulatory period and the subsequent ability of the male to copulate (Hull et al., 1995) was observed in the present experiment. A period of 2 days of systemic testosterone replacement in long-term castrates was not sufficient for restoration of either sexual behavior or the increase in DA release typically seen in the MPOA of male rats upon exposure to an estrous female. Five days of systemic testosterone administration, however, was sufficient for the recovery of sexual behavior and the MPOA DA response in most castrates in which copulation had been extinguished. Complete recovery of copulation and the MPOA DA response was observed in all animals treated with systemic testosterone for 10 days. Thus, 5 days appears to be the threshold period for hormone replacement to restore the DA response and copulatory behavior.

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