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Castration decreases extracellular, but increases intracellular, dopamine in medial preoptic area of male rats

Jianfang Du, Daniel S. Lorrain, Elaine M. Hull *

Department of Psychology, Park Hall, State University of New York at Buffalo, Buffalo, NY 14260, USA

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Abstract

Dopamine (DA) is released in the medial preoptic area (MPOA) of male rats in the presence of a female, and it facilitates male sexual behavior. Castration blocks the DA response to a female and the male's ability to copulate. The present experiments examined the effects of castration on (1) basal levels of extracellular DA in the MPOA, using the no net flux microdialysis technique, (2) the response of extracellular DA to amphetamine, and (3) tissue levels of DA. Castrated rats had lower basal levels of extracellular DA in the MPOA, compared with gonadally intact rats; in vivo recovery, a measure of uptake, was not different. This suggests that castration decreases DA release in basal conditions, as well as in response to a female. However, systemic amphetamine injections, which induce DA release, resulted in greater DA release in castrates. Finally, tissue levels of DA were higher in the MPOA, the caudate–putamen and the bed nucleus of stria terminalis of castrates. These data suggest that DA synthesis and storage in the MPOA are normal, or even enhanced, in castrates, and uptake is not altered. The deficit in extracellular levels appears to be related to release, perhaps due to decreased nitric oxide. © 1998 Elsevier Science B.V.

Keywords: Male rat; Castration; Dopamine; Amphetamine; Medial preoptic area; Bed nucleus of stria terminalis; Caudate putamen; Amygdala; Microdialysis

1. Introduction

Dopamine (DA) released in the medial preoptic area (MPOA) facilitates male rat sexual behavior. Specifically, the classic DA agonist apomorphine infused into the MPOA increased the rate and efficiency of copulation [13], whereas infusion of the DA antagonist *cis*-flupenthixol into the MPOA impaired copulation, penile reflexes and sexual motivation [28]. Furthermore, in vivo microdialysis revealed an increase in extracellular DA in the MPOA, when a sexually receptive female was presented behind a barrier, and a further increase during the copulatory period [14].

Male rat copulatory behavior is hormone-dependent. Following castration, males gradually lose their copulatory ability [6]. Systemic injections of testosterone can restore copulatory behavior [2], but it takes about 10 days after the onset of treatment to restore the behavior fully [20]. Furthermore, a recent in vivo microdialysis study showed that none of the males that had been castrated for 2 weeks displayed a DA response to the sexually active female behind the barrier, as the gonadally intact rats had, nor did they copulate when the barrier was removed [14]. Thus, it was hypothesized that testosterone may serve as a permissive factor for the DA release in response to a receptive female [14].

The present study investigated whether the decrease in extracellular DA in castrates is also observed in basal conditions, or only in a sexual context. It also tested whether the decreased extracellular levels resulted from decreased synthesis, increased uptake, or decreased release. The quantitative in vivo microdialysis technique (the 'no net flux' method), which provides a more accurate estimate of the extracellular DA concentrations than standard microdialysis procedures [22], was used in the first experiment to compare the extracellular DA levels in the MPOA of intact and castrated rats. This method uses different amounts of DA added to the dialysate; if the dialysate contains more DA than the tissue, some of it will diffuse into the brain and the loss will be detectable. On the other hand, if the brain contains more DA than the dialysate, DA will diffuse into the dialysate, and the increase can be measured. A regression line is drawn, plotting the loss or gain of DA for each concentration of

* Corresponding author. Fax: +1 716 6453801; E-mail: emhull@acsu.buffalo.edu

DA in the dialysate; the point at which the line crosses from loss to gain (the point of no net flux into or out of the probe) is taken as the extracellular level of DA. In Expt. 2, DA responses to systemic injections of amphetamine were compared in castrated and gonadally intact rats, using in vivo microdialysis. Amphetamine releases DA from storage vesicles and also reverses the DA transporter [27], and can therefore be used to measure previously synthesized and stored DA. In the third experiment, the tissue punch method was used to estimate the total DA content in the MPOA, the bed nucleus of the stria terminalis (BNST), the caudate–putamen (CP) and the amygdala of gonadally intact and castrated rats. All these four brain areas are directly or indirectly involved in regulating male rat sexual behavior.

2. Materials and methods

2.1. Subjects

Adult male Long-Evans/Blue Spruce rats (about 300 g at the time of surgery) were obtained from Harlan Sprague-Dawley (Indianapolis, IN). They were housed individually in large plastic cages. A 14:10-h light/dark cycle was in effect, with lights out at 11.00 h. Food and water were available ad libitum. Twenty-two animals were castrated 1 week after they arrived; 17 gonadally intact males served as controls.

2.2. Surgical procedures

2.2.1. Castration

Animals were anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4 mg/kg). A longitudinal midscrotal incision was made, and the testes and surrounding fatty tissue were tied off. A cut was made distal to the ligature, to remove the testes and surrounding tissue; the skin was sutured and spread with bacitracin ointment. For Expt. 1, eight castrated animals were allowed 2 weeks to recover before intracranial implantation. The same animals were used in Expt. 2, 1 week later. For Expt. 3, the remaining 14 animals were sacrificed 3 months after they were castrated.

2.2.2. Intracranial implantation

Two weeks before undergoing microdialysis in Expt. 1, eight castrated and eight intact animals were anesthetized with 50 mg/kg ketamine hydrochloride and 4 mg/kg xylazine hydrochloride. They were then implanted with a guide cannula, made of 23-gauge thin-wall stainless steel. The cannula ended 1.5 mm above the MPOA (AP, 2.4 mm; ML, 0.2 mm; DV, −6.3 mm; incisor bar, +5.0; according to [23]) and was secured to the skull and skull screws with dental acrylic. Microdialysis probes were designed to extend 3 mm beyond the tip of the guide cannula. A male connector and a removable microdialysis set were also embedded in the dental cement. The male connector was attached to a female connector on a swivel and provided stability of the probe during movement of the animal. An obturator, cut the same length as the guide cannula, was inserted into the guide cannula until the day of testing. After the dental acrylic was hardened around the probe assembly, bacitracin antibiotic was spread around the wound margins, and the animal was injected with gentamicin antibiotic (0.02 mg/kg/day for 3 days).

2.3. Apparatus

Concentric microdialysis probes were constructed according to the method of Yamamoto and Pehek [29]. The probe consisted of 27-gauge thin-wall stainless-steel tubing, to which 3 mm of dialysis membrane (MW cut-off 6000; 210 μm o.d.; Spectra-Por) was epoxied. The active dialyzing surface of the membrane was reduced to 1 mm with waterproof epoxy. Dialysate flowed in through PE 20 inlet tubing and out through a concentric silica capillary tube (150 μm o.d.) and was collected into a 250-μl centrifuge tube.

The LC Packings (San Francisco, CA) capillary chromatographic system consisted of an Acurate microflow processor and pulse-damper, a Rheodyne injector with a 500-nl sample loop, and an Antec micro electrochemical detector, equipped with a microflow cell (11 nl cell volume), with a glassy carbon working electrode maintained at an applied potential of +0.7 V relative to the Ag/AgCl reference electrode. The analytical column was an LC Packings Fusica reversed phase capillary column (320 μm i.d., 5 cm long, packed with 3 μm C-18 particles). A Gilson model 307 pump delivered mobile phase through the system at 0.50 ml/min; however, the Acurate microflow processor split the flow, so that flow through the analytical column was approximately 7 μl/min. The mobile phase consisted of 32 mM citric acid, 54.3 mM sodium acetate, 0.074 mM Na_2 EDTA, 0.215 mM octane sulfonic acid (OSA), 3% methanol (v/v), and 0.004% tetrahydrofuran. The mobile phase was filtered and degassed under vacuum; pH was 3.8. Data were collected using a Gateway 2000 486 microcomputer workstation running Gilson 715 HPLC system controller software, which also controlled the pump parameters.

2.4. Experimental procedures

Experiment 1 compared the basal extracellular DA concentrations in the MPOA of gonadally intact and castrated male rats, using the no net flux method. Eight gonadally intact males and eight 1-month castrates were used. On the

day of testing, animals were lightly anesthetized with ketamine hydrochloride (25 mg/kg) and xylazine hydrochloride (2 mg/kg) to allow the dialysis probe to be inserted into the MPOA. Dulbecco's phosphate-buffered saline (Sigma Chemical, St. Louis, MO) (138 mM NaCl, 2.7 mM KCl, 0.5 mM $MgCl_2$, 1.5 mM KH_2PO_4, 1.2 mM $CaCl_2$, pH 6.5) was perfused at a rate of 0.5 μl/min with a Harvard model #22 infusion pump, using a 1-ml gastight syringe. Approximately 4 h after probe insertion, perfusate samples were collected at 6-min intervals, and 3-μl samples were injected onto the capillary HPLC system. After a stable basal DA level was reached (variation of the last three samples under 10%), the perfusate was changed in a random sequence to a Dulbecco's solution containing no DA and at least three of the following DA concentrations: 0.817, 3.268, 6.536, 13.072 nM. A 45-min equilibrium period (determined by our pilot data) was required after switching the perfusate, and then three 6-min samples were collected for each DA concentration in the perfusate. At the end of the experiment, the probe was removed and the obturator was inserted back into the cannula. One intact animal was sacrificed after Expt. 1, because its guide cannula assembly became detached. The remaining 15 animals were kept for Expt. 2.

Simple linear regressions were used to obtain slope and intercept values for each animal. Gain or loss of DA to or from the probe (the difference between the concentration of DA added to the dialysate and the average concentration in three 6-min samples, after passing through the probe) was plotted as a function of the concentration of DA added to the dialysate. The *x*-axis value that corresponded to the zero intercept on the *y*-axis was taken as the basal extracellular DA concentration.

Experiment 2 examined the MPOA DA response to systemic amphetamine injections in castrated and gonadally intact rats, using in vivo microdialysis. Eight castrated and seven intact rats from Expt. 1 were reused in this experiment after a 1-week recovery period. On the day of testing, animals were lightly anesthetized, and the concentric dialysis probes were inserted into the MPOA as in Expt. 1. After a stable baseline was obtained (about 4 h), the rat received an i.p. injection of amphetamine sulfate (1.0 mg/kg). This dosage was chosen because it significantly elevated extracellular DA in the ventral striatum [12]. Eight postinjection samples were collected at 10-min intervals and analyzed.

In Expt. 3, total DA content in tissue punches from the MPOA, BNST, CP, and amygdala of intact and castrated rats was estimated. Nine gonadally intact males and 14 3-month castrates were used. On the day of the experiment, animals were sacrificed, and brains were removed and immediately frozen. Tissue was punched from 1000-μm sections of the above areas, using an 18-gauge stainless-steel tube. Tissue was then weighed and put into 0.1 M perchloric acid solution. Samples were homogenized and centrifuged at 10 000 rpm for 30 min. The supernatant of the solution was then drawn off, filtered, and injected into the HPLC system. Amounts of DA per 100 μg of tissue from castrated vs. intact rats were compared for each of the four brain areas.

2.5. Histology

At the end of Expt. 2, probe placements were verified histologically. The animal was deeply anesthetized with pentobarbital, and Evans blue stain was perfused through the probe. After decapitation of the animal, the brain was removed, frozen, and cut with a Cryocut microtome into 40-μm slices, which were dry-mounted onto slides. Sections were examined with a projection magnifier. Data from animals (two intact and three castrated rats) with probes located outside the MPOA were not included in statistical analysis.

2.6. Statistics

For data in Expt. 1, the extracellular DA concentration and the regression slope (in vivo recovery of DA) were compared between gonadally intact and castrated animals by *t*-test.

In Expt. 2, chromatographic data (height of the curve for DA and DOPAC) from nine samples (last baseline and eight post-injection samples) were subjected to two-factor (gonadal condition and time course) repeated measures analyses of variance, followed by Newman–Keuls multiple comparisons tests. One-way repeated measures analyses of variance were also used for individual analysis of gonadally intact and castrated groups.

In Expt. 3, *t*-tests were used to compare the amount of DA in MPOA, BNST, CP and amygdala tissue punches from intact vs. castrated rats.

3. Results

3.1. Experiment 1: extracellular DA levels in the MPOA

The no net flux method revealed that basal extracellular DA levels in the MPOA of intact animals were significantly higher than in castrates ($t_{(9)} = 2.62$, $P < 0.05$). Gonadally intact rats had a mean DA extracellular concentration of 1.394 ± 0.377 nM (mean ± S.E.M., $n = 6$), whereas castrates had 0.235 ± 0.165 nM ($n = 5$) (Fig. 1).

There was no significant difference of in vivo recovery of DA between gonadally intact and castrated rats ($t_{(9)} = 0.416$, not significant (n.s.)). For gonadally intact rats, the in vivo recovery of DA (the slope of the regression) was $59.2 \pm 5.7\%$ (Fig. 1). For castrated rats, the in vivo recovery of DA was $64.1 \pm 11\%$ (Fig. 1). In vivo recovery is considered to be a measure of uptake [21].

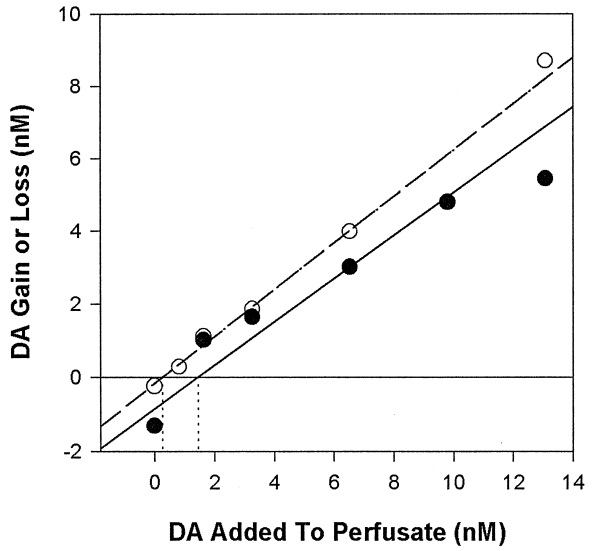

Fig. 1. Mean loss or gain of DA to or from the brain as a function of the perfusate DA concentration. The no net flux method of microdialysis was used to determine the extracellular levels of DA in the MPOA of gonadally intact ($n = 6$) and castrated males ($n = 5$). The dialysate contained concentrations of DA as indicated on the abscissa; mean loss of DA from the dialysate or gain from the brain to the dialysate are plotted as a function of the concentration of DA in the dialysate. The point at which the regression line crosses the abscissa (no loss or gain of DA) is taken as the level of extracellular DA. The slope of the regression line is taken as the in vivo recovery of DA to or from the brain and is thought to be a measure of uptake. The solid line is the mean regression line for gonadally intact animals. The broken line is the mean regression line for animals castrated one month previously. Extracellular DA levels in the MPOA were significantly higher in gonadally intact males than in males castrated 1 month previously. In vivo recovery rates, which are thought to be a measure of uptake, did not differ between gonadally intact and castrated animals.

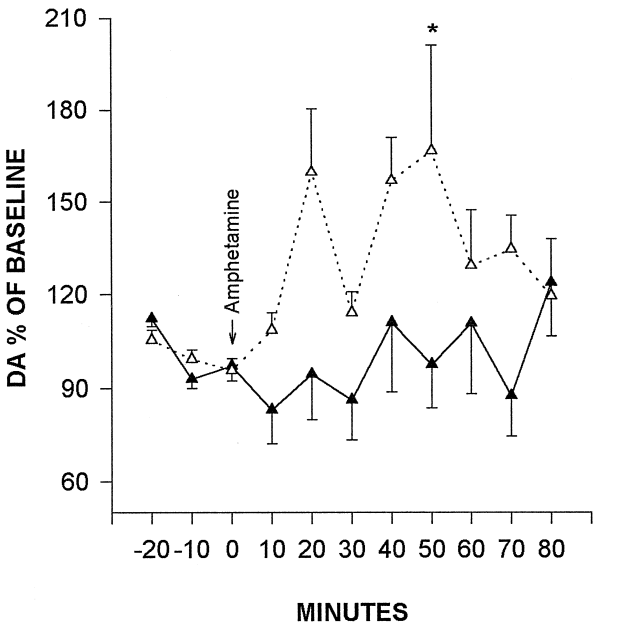

Fig. 2. Amphetamine-stimulated levels of extracellular DA in the MPOA of gonadally intact ($n = 5$) and castrated male rats ($n = 5$). Following a systemic injection of amphetamine (1 mg/kg), extracellular DA levels rose only in castrated (open triangles), and not in gonadally intact (black triangles) rats. In castrates DA levels were significantly higher than baseline in the 40–50 min postinjection sample, $^{*}P < 0.05$.

3.2. Experiment 2: MPOA DA responses to amphetamine challenge

Amphetamine injections (1.0 mg/kg, i.p.) increased extracellular DA in the MPOA of castrated ($F_{8,32} = 3.04$, $P < 0.05$), but not gonadally intact rats ($F_{8,32} = 1.02$, n.s.) (Fig. 2). Two-way repeated measures of analyses of variance revealed that the main effect of gonadal condition was significant ($F_{1,8} = 5.36$, $P < 0.05$), as was the main effect of time course ($F_{8,64} = 2.36$, $P < 0.05$). There was a trend for the interaction between these two factors to be significant ($F_{8,64} = 1.82$, $P = 0.09$). Multiple comparisons, using Newman–Keuls tests, showed that DA levels were significantly increased in the castrates 40–50 min following amphetamine injection, compared to baseline (Fig. 2).

Following amphetamine injection, DOPAC levels were significantly decreased in both gonadally intact ($F_{8,32} = 6.44$, $P < 0.001$) and castrated rats ($F_{8,32} = 3.19$, $P < 0.001$) (Fig. 3). Two-way repeated measures analysis of variance showed that the main effect of gonadal condition was not significant ($F_{1,8} = 4.026$, n.s.), whereas the main effect of time course was significant ($F_{8,64} = 9.733$, $P <$

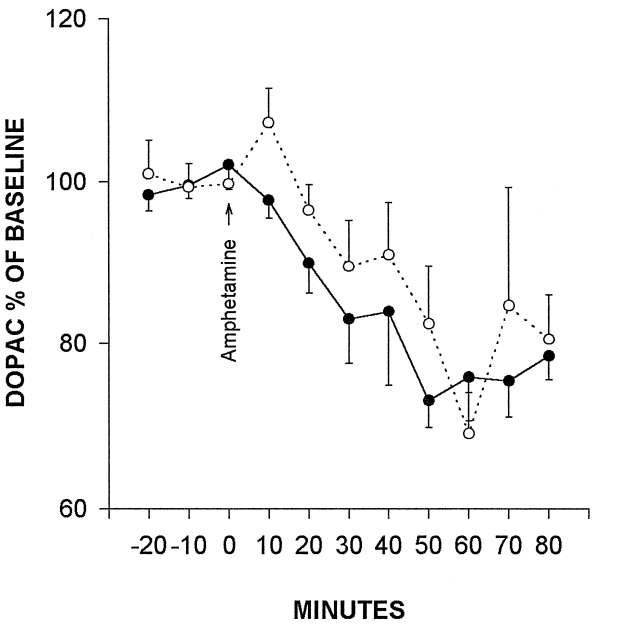

Fig. 3. Amphetamine-stimulated levels of extracellular DOPAC in the MPOA of gonadally intact ($n = 5$) and castrated male rats ($n = 5$). A systemic injection of amphetamine (1 mg/kg) resulted in a significant decrease in DOPAC levels ($P < 0.001$) in both gonadally intact (black circles) and castrated (open circles) male rats. There was no significant difference between the two groups.

Table 1
DA content in tissue punches from the medial preoptic area (MPOA), bed nucleus of stria terminalis (BNST), caudate putamen (CP) and amygdala

Site	Condition	DA, pg/100 ug tissue weight (mean ± S.E.M.)	Sample size
MPOA	Castrated	192.29 ± 39.57 *	14
	Intact	82.31 ± 22.67	9
BNST	Castrated	651.78 ± 100.37 *	12
	Intact	303.25 ± 90.88	9
CP	Castrated	44 390 ± 3200 *	9
	Intact	22 330 ± 2930	4
Amygdala	Castrated	413.10 ± 152.81	14
	Intact	102.82 ± 21.08	9

Castrates, compared to gonadally intact males, had significantly more DA in tissue punches from the MPOA, BNST and CP. The apparent increase in the amygdala of castrates was not statistically significant.
* $P < 0.05$.

0.001). There was no interaction between these two factors ($F_{8,64} = 0.809$, n.s.).

3.3. Experiment 3: DA content in MPOA, BNST, CP and amygdala tissue

Castrated rats had significantly more DA content in tissue punches from the MPOA ($t_{(21)} = 2.48$, $P < 0.05$), BNST ($t_{(19)} = 2.48$, $P < 0.05$) and CP ($t_{(11)} = 2.48$, $P < 0.01$) (Table 1). Castration did not significantly affect DA content in tissue from the amygdala ($t_{(21)} = 1.61$, $P = 0.1226$) (Table 1).

4. Discussion

Using the no net flux method, this study showed that extracellular DA concentrations in the MPOA were higher in gonadally intact than in castrated rats. However, more DA was released in the castrated, than in the gonadally intact rats, when amphetamine was injected systemically. Amphetamine depletes vesicular stores of DA and reverses the membrane DA transporter [27], thereby inducing the release of stored DA. The time of greatest increase of DA following systemic injection of amphetamine in castrates was 40–50 min, which is approximately the same as the previous findings in the ventral striatum [21,12]. On the other hand, the quantity of DA released, as a percent of baseline, is much lower in MPOA than in ventral striatum, probably because there are very few DA transporters in the hypothalamus and MPOA [5]. These data suggest that castrates have more DA available for release than do intact males. This interpretation is supported by analyses of tissue punches, which contain primarily intracellular transmitter; castrates had significantly greater DA in MPOA, BNST and CP tissue than did gonadally intact rats. Therefore, castrates appear to have more DA stored in the terminal, but less released into the synapse, than do intact rats.

We recently reported that intact males, and most 1-week castrates, had increased extracellular DA in the MPOA in response to an estrous female behind a barrier and during copulation after the barrier was removed [14]. The remaining 1-week castrates and all 2-week castrates failed to show the DA response to the female and failed to copulate. Therefore, if DA can be released, it may facilitate copulatory behavior in castrates, as well as in intact rats. This finding is consistent with previous demonstrations that MPOA microinjections of the classic DA agonist apomorphine partially restored copulation in long term castrates [25], and also increased copulatory rate and efficiency of intact males [13]. The present study showed that extracellular DA in the MPOA was lower in castrates during basal conditions, as well as in response to a female, as described above [14].

Decreased extracellular levels of DA in the MPOA of castrates could theoretically result from decreased synthesis, decreased release and/or increased uptake. A decrease in synthesis is unlikely, since there were higher levels of DA in tissue punches from castrated, compared with intact rats, and since amphetamine elicited greater DA release in the MPOA of castrates. Indeed, the lower extracellular DA levels in castrates could result in enhancement of synthesis, since inhibitory autoreceptors would receive less stimulation.

Increased uptake also seems not to explain the lower extracellular DA levels in castrates. In vivo recovery of DA (slope of the regression lines) in Expt. 1 was not different for the two groups. In vivo recovery rate reflects the dynamics of neurotransmitter release and clearance, that is, the efficiency of neurotransmitter being taken to the brain from the perfusate and neurotransmitter being released from brain into the perfusate. It has been suggested that in vivo recovery is more likely to be controlled by concentration-dependent processes, such as uptake, than by non-concentration-dependent processes, such as release, according to the theoretical model of in vivo dialysis [22]. In addition, DOPAC levels declined similarly in castrates and intact animals following amphetamine administration in Expt. 2. A major source of DOPAC is previously released DA, which is transported back into the terminal and converted to DOPAC by monoamine oxidase (MAO). Although amphetamine may inhibit MAO, its main effect is to deplete vesicular stores and to reverse the transporter, thereby releasing stored DA and inhibiting uptake [27]. The decline in DOPAC levels in both castrates and intact males suggests that (1) amphetamine did, indeed, inhibit uptake, and (2) that castration did not affect this process. Therefore, the lower extracellular DA levels in castrates during basal conditions appear not to be related to changes in uptake.

The most likely explanation for the decreased extracellular DA in the MPOA of castrates is decreased release.

Although neuronal firing is undoubtedly one factor regulating DA release, another factor that may be important in regulating DA release in the MPOA is nitric oxide (NO). NO has been reported to increase the release of DA from striatal slices [10,17,30]. The NO precursor, L-arginine, administered into the MPOA via a microdialysis probe, increased extracellular levels of DA [18]. Furthermore, the synthetic enzyme, nitric oxide synthase (NOS) may be hormonally regulated. In the male Syrian hamster, castration reduced NOS-positive neurons in the medial preoptic nucleus [11]. We recently reported that castrated male rats also had significantly fewer NOS-positive neurons in the MPOA than did gonadally intact rats [8]. Thus, the effects of gonadal steroid hormones on DA release in the MPOA may be through their actions on NOS. That is, following castration, when virtually no androgen or its receptor is present in the brain [16], the amount of NOS is reduced; this results in decreased release of DA. The importance of NO for DA release in the MPOA was recently confirmed; a NOS inhibitor, administered via reverse dialysis into the MPOA, blocked DA release during copulation [19]. Because DA release was inhibited only in the immediate vicinity of the probe, the animals were still able to copulate.

The present study also tested whether castration affected DA levels in several other brain areas that have been implicated in the control of sexual behavior. Tissue DA levels were significantly higher in punches from MPOA, BNST and CP of castrates, compared with intact males; DA levels in the amygdala of castrates were non-significantly elevated, as well. This suggests that castration may affect DA levels in numerous brain areas that are directly or indirectly involved in male rat sexual behavior. It is not clear how widespread this effect may be. There are inconsistencies in the literature regarding the effects of castration on various measures of DA activity. In the ventral striatum, a site where DA release is correlated with copulation in male rats [24], castration increased amphetamine-stimulated levels of extracellular DA [12], similar to the effects in the present study. On the other hand, castration was reported to decrease tissue levels of DA and DOPAC in ventral striatum [1]. In the dorsal striatum, castration did not affect measures of DA turnover or amphetamine-stimulated DA release [1,3,4]. However, castration was reported to increase DA efflux from the dorsal striatal tissue slices [7]. In the MPOA, castration was reported to increase [26] or decrease [9] DA levels or turnover. Some of these inconsistencies may have resulted from differences in technique, time since castration, or brain location. Therefore, it is difficult to discern common effects of castration on DA levels or release across brain areas. However, the current and previous [14] results from our laboratory are internally consistent in showing decreased extracellular DA levels in the MPOA of castrates, both in basal conditions and in a sexual context, and increased intracellular DA in the MPOA.

The present study may help to explain two seemingly opposite behavioral effects of castration [6]. On the one hand, mount and intromission latencies increased steadily in the weeks following castration. On the other hand, if castrates did initiate copulation, they ejaculated with shorter latencies and fewer intromissions. According to the present study, one explanation may be that it is harder for MPOA DA to be released in the castrates; but if DA can be released, castrates have more stored DA, which may facilitate ejaculation more readily than before castration. This suggestion is consistent with the finding that high doses of DA agonists microinjected into the MPOA facilitated ejaculation, apparently via stimulation of D_2-like receptors [15].

In summary, castration decreases extracellular DA concentration during basal conditions and in response to a female. These decreased levels primarily result from decreased release, rather than inhibited synthesis or increased uptake. The inhibition of DA release may be related to the increased latency to copulate after castration. However, castrates have more DA stored in the terminal, which, if released, may contribute to the decreased ejaculatory threshold following castration.

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References

[1] L.M. Alderson, M.J. Baum, Differential effects of gonadal steroids on dopamine metabolism in mesolimbic and nigro-striatal pathways of male rat brain, Brain Res. 218 (1981) 189–206.
[2] F.A. Beach, A.M. Holz-Tucker, Effects of different concentrations of androgen upon sexual behavior in castrated male rats, J. Comp. Physiol. Psychol. 42 (1949) 433–453.
[3] J.B. Becker, V.D. Ramirez, Sex differences in the amphetamine stimulated release of catecholamines from rat striatal tissue in vitro, Brain Res. 204 (1980) 361–372.
[4] J.B. Becker, V.D. Ramirez, Experimental studies on the development of sex differences in the release of dopamine from striatal tissue fragments in vitro, Neuroendocrinology 32 (1981) 168–173.
[5] B.J. Ciliax, C. Heilman, L.L. Demchyshyn, Z.B. Pristupa, E. Ince, S.M. Hersch, H.B. Niznik, A.I. Levey, The dopamine transporter: immunochemical characterization and localization in brain, J. Neurosci. 15 (1995) 1714–1723.
[6] J.M. Davidson, Characteristics of sex behavior in male rats following castration, Animal Behav. 14 (1966) 266–272.
[7] D.E. Dluzen, V.D. Ramirez, Effects of orchidectomy on nigrostriatal dopaminergic function: Behavioral and physiological evidence, J. Neuroendocrinol. 1 (1989) 285–290.
[8] J. Du, L.A. Lumley, E.M. Hull, Effects of castration and hormone replacement on nitric oxide synthase in the medial preoptic area of male rats, Abstr. Soc. Neurosci. 22 (1996) 1414.
[9] J.W. Gunnet, K.J. Lookingland, K.E. Moore, Comparison of the effects of castration and steroid replacement on incertohypothalamic

dopaminergic neurons in male and female rats, Neuroendocrinology 44 (1986) 269–275.
[10] I. Hanbauer, D. Wink, Y. Osawa, G.M. Edelman, J.A. Gally, Role of nitric oxide in NMDA-evoked release of [^{3}H]-dopamine from striatal slices, NeuroReport 3 (1992) 409–412.
[11] Y. Hadeishi, R.L. Wood, Nitric oxide synthase in mating behavior circuitry of the male Syrian hamster brain, J. Neurobiol. 30 (1996) 480–492.
[12] L. Hernandez, L. Gonzalez, E. Murzi, X. Paez, E. Gottberg, T. Baptista, Testosterone modulates mesolimbic dopaminergic activity in male rats, Neurosci. Lett. 171 (1994) 172–174.
[13] E.M. Hull, D. Bitran, E.A. Pehek, R.K. Warner, L.C. Band, G.M. Holmes, Dopaminergic control of male sex behavior in rats: Effects of an intracerebrally infused agonist, Brain Res. 370 (1986) 73–81.
[14] E.M. Hull, J. Du, D.S. Lorrain, L. Matuszewich, Extracellular dopamine in the medial preoptic area: Implications for sexual motivation and hormonal control of copulation, J. Neurosci. 15 (1995) 7465–7471.
[15] E.M. Hull, R.C. Eaton, V.P. Markowski, J. Moses, L.A. Lumley, J.A. Loucks, Opposite influence of medial preoptic D_1 and D_2 receptors on genital reflexes: Implications for copulation, Life Sci. 51 (1992) 1705–1713.
[16] L.C. Krey, M.Y. McGinnis, Time-courses of the appearance/disappearance of nuclear androgen + receptor complexes in the brain and adenohypophysis following testosterone administration/withdrawal to castrated male rats: Relationships with gonadotropin secretion, J. Steroid Biochem. 35 (1990) 403–408.
[17] G. Lonart, K.L. Cassels, K.M. Johnson, Nitric oxide induces calcium-dependent [^{3}H]dopamine release from striatal slices, J. Neurosci. Res. 35 (1993) 192–198.
[18] D.S. Lorrain, E.M. Hull, Nitric oxide increases dopamine and serotonin release in the medial preoptic area, NeuroReport 5 (1993) 87–89.
[19] D.S. Lorrain, L. Matuszewich, R.V. Howard, J. Du, E.M. Hull, Nitric oxide promotes medial preoptic dopamine release during male rat copulation, NeuroReport 8 (1996) 31–34.
[20] M.Y. McGinnis, R.M. Dreifuss, Evidence for a role of testosterone-androgen receptor interactions in mediating masculine sexual behavior in male rats, Endocrinology 124 (1989) 618–626.
[21] R.J. Olson, J.B. Justice Jr., Quantitative microdialysis under transient conditions, Anal. Chem. 65 (1993) 1017–1022.
[22] L.H. Parsons, J.B. Justice, Quantitative approaches to in vivo brain microdialysis, Crit. Rev. Neurobiol. 8 (1994) 189–220.
[23] L.J. Pellegrino, A.S. Pellegrino, A.J. Cushman, A Stereotaxic Atlas of the Rat Brain, 2nd ed., Plenum, New York, 1979.
[24] J.G. Pfaus, A.G. Phillips, Role of dopamine in anticipatory and consummatory aspects of sexual behavior in the male rat, Behav. Neurosci. 105 (1991) 727–743.
[25] L.L. Scaletta, E.M. Hull, Systemic or intracranial apomorphine increases copulation in long-term castrated male rats, Pharmacol. Biochem. Behav. 37 (1990) 471–475.
[26] J.W. Simpkins, P.S. Kalra, S.P. Kalra, Inhibitory effects of androgens on preoptic area dopaminergic neurons in castrated rats, Neuroendocrinology 31 (1980) 177–181.
[27] D. Sulzer, J.K. Chen, Y.Y. Lau, H. Kristensen, S. Rayport, A. Ewing, Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport, J. Neurosci. 15 (1995) 4102–4108.
[28] R.K. Warner, J.T. Thompson, V.P. Markowski, J.A. Loucks, T.J. Bazzett, R.C. Eaton, E.M. Hull, Microinjection of the dopamine antagonist cis-flupenthixol into the MPOA impairs copulation, penile reflexes and sexual motivation in male rats, Brain Res. 540 (1994) 177–182.
[29] B.L. Yamamoto, E.A. Pehek, A neurochemical heterogeneity of the rat striatum as measured by in vivo electrochemistry and microdialysis, Brain Res. 506 (1990) 236–242.
[30] X.Z. Zhu, L.G. Luo, Effect of nitroprusside (nitric oxide) on endogenous dopamine release from rat striatal slices, J. Neurochem. 59 (1992) 932–935.