available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Getting his act together: Roles of glutamate, nitric oxide, and dopamine in the medial preoptic area

Elaine M. Hull, Juan M. Dominguez*

Department of Psychology and Program in Neuroscience, Florida State University, Tallahassee, FL 32306-1270, USA

ARTICLE INFO

Article history:
Accepted 4 August 2006
Available online 11 September 2006

Keywords:
Copulation
Rat
Experience
Sensitization
Gonadal hormone

ABSTRACT

Gonadal hormones have primarily slow, genomically mediated effects, but copulation requires rapid interactions with a partner. A major way in which hormones facilitate male sexual behavior is by increasing production of neurotransmitter receptors or of enzymes that regulate neurotransmitter synthesis or release. Dopamine is an important facilitative neurotransmitter, and the medial preoptic area (MPOA) is a critical integrative site for male sexual behavior. MPOA dopamine is released before and during mating and facilitates copulation, genital reflexes, and sexual motivation. Gonadal hormones regulate dopamine release in the MPOA of male rats in part by increasing nitric oxide synthase (NOS) in the MPOA; the resultant increase in production of nitric oxide (NO) increases both basal and female-stimulated dopamine release. Glutamate also increases dopamine release via increased production of NO. At least some of the glutamatergic inputs to the MPOA are from the medial amygdala (MeA) and bed nucleus of the stria terminalis (BNST), which mediate the female-stimulated increase in dopamine, which in turn enhances copulatory ability. Extracellular glutamate in the MPOA increases during copulation, especially during ejaculation, and increased glutamate facilitates copulation and genital reflexes. Previous sexual experience also facilitates copulation and confers resistance to impairment by various lesions, drugs, and stress. Experience enhances processing of sexual stimuli, and its effects require activation of glutamate NMDA receptors and NOS in the MPOA. Neuronal NOS is increased in the MPOA of experienced males. Therefore, glutamate, NO, and dopamine interact in the MPOA to facilitate mating and to enhance future sexual responsiveness.

1. Introduction

Male sexual behavior is highly dependent on gonadal hormones, which have primarily (though not exclusively) slow, genomically mediated effects (reviewed in Hull et al., 2006). However, copulation requires rapid somatomotor interactions between two active individuals. The means by which the slow hormonal effects are translated into rapid, interactive behaviors includes the up-regulation of various enzymes and receptors that allow neurotransmitters to activate (or inhibit) specific neurons that integrate and execute the behaviors. This review will summarize research, primarily from our laboratory, that sheds light on the means by which hormones activate male rat sexual behavior and the means by which previous sexual experience enhances the ability to copulate. Sexually experienced male rats show increased preference for being with a receptive female (Lopez et al., 1999), require less time and stimulation to achieve ejaculation (Dewsbury, 1969; Larsson, 1959; Pfaus and Wilkins, 1995), and are more resistant to impairments due to various lesions (Claro et al., 1995; de Jonge

* *Corresponding author*. Fax: +1 850 644 7739.
E-mail address: hull@psy.fsu.edu (E.M. Hull).

0006-8993/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2006.08.031

et al., 1989; Kondo, 1992; Saito and Moltz, 1986) and stress (Pfaus and Wilkins, 1995). Even repeated exposures to a female, without actual mating, is sufficient to enhance copulatory ability (Lagoda et al., 2004; Powell et al., 2003; see below). We will explore both the normal neural events that lead to the behavior and the means by which experience enhances its execution.

2. The neural circuitry of male sexual behavior

The neural connections that coordinate and execute sexual behaviors begin with chemosensory input from the main olfactory and vomeronasal systems and with somatosensory input from the genitals. Auditory input, especially ultrasonic vocalizations, also enhance the likelihood of mating (Barfield and Thomas, 1986), but the roles of those stimuli are less well studied. Chemosensory efferents from the main and accessory olfactory systems project to the medial amygdala (MeA), which in turn relays information to the medial preoptic area (MPOA) both directly and indirectly, via efferents to the bed nucleus of the stria terminalis (BNST) (see Fig. 1). Some of the neurons of the chemosensory pathways contain nuclear steroid receptors, and there are rich interconnections with an adjacent hormonal circuit; therefore, gonadal hormones can influence the receipt and processing of chemosensory stimuli (reviewed in Wood and Newman, 1995; Simerly, 1995). Genitosensory input, relayed from the spinal cord to the central tegmental field (CTF, sometimes referred to as the dorsolateral tegmentum), also projects to both the MPOA and the MeA (Baum and Everitt, 1992; Simerly and Swanson, 1986). A component of the CTF, the parvocellular portion of the subparafascicular nucleus (SPFp), seems to be especially important for stimuli related to ejaculation (reviewed in Coolen et al., 2004; Hull et al., 2006). The MPOA sends efferents to the paraventricular nucleus of the hypothalamus (PVN), the ventral tegmental area (VTA, the source of the mesocorticolimbic pathway), the nucleus paragigantocellularis (nPGi, a major source of tonic inhibition on genital reflexes), and other autonomic and somatomotor areas.

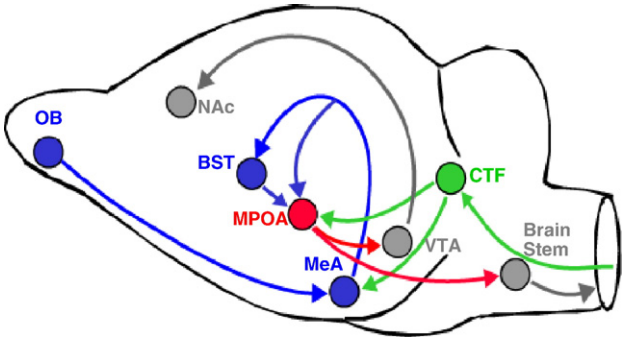

Fig. 1 – Neural circuits regulating male sexual behavior. The medial preoptic area (MPOA) receives direct and indirect input from brain areas that are important for the assimilation of sexually relevant information. Olfactory stimulation is received by the olfactory bulbs (OB), the OB project to the medial amygdala (MeA), which relays information to the bed nucleus of stria terminalis (BST) and the MPOA. Additionally, the MPOA and MeA receive somatosensory input via the central tegmental field (CTF). In turn, the MPOA projects to the ventral tegmental area (VTA) and the brain stem (BS). For a complete description of MPOA afferents and efferents, see Simerly and Swanson (1986, 1988).

3. The role of MPOA dopamine in male sexual behavior and the effects of sexual experience

The MPOA is the main integrative area for male sexual behavior. Large lesions of the MPOA abolish copulatory ability, and electrical or chemical stimulation facilitates mating and elicits genital reflexes (reviewed in Hull et al., 2006). Compared to previously naive males, sexually experienced males showed a greater increase in immunoreactivity for the protein product of the immediate-early gene *c-fos* (Fos-ir) in the MPOA following copulation to one ejaculation (Lumley and Hull, 1999) (see Fig. 2). The increase was even more notable in that experienced males ejaculated after fewer vaginal intromissions, compared to naive males. Thus, the MPOA of experienced males appeared to have been sensitized to sexual stimuli, with fewer intromissions required to trigger ejaculation and fewer copulatory behaviors eliciting more Fos-ir. The dopamine D_1 receptor antagonist SCH-39166 decreased the number of mating-induced Fos-ir cells and also inhibited some copulatory measures in sexually naive males, though not in experienced males (Lumley and Hull, 1999). This is consistent with previous studies, noted above, showing increased resistance of experienced males to impairment by various lesions, stress, and castration.

One neurotransmitter in the MPOA that facilitates male sexual behavior is dopamine. Microinjections of dopamine agonists into the MPOA facilitate copulation and genital reflexes, whereas dopamine antagonists impair copulation, genital reflexes, and sexual motivation (reviewed in Dominguez and Hull, 2005; Hull et al., 2006). Briefly, low doses of a D_2/D_3 agonist (quinelorane) decreased the latency to the first *ex copula* genital reflex without affecting the numbers of reflexes; however, high doses of quinelorane inhibited *ex copula* erections but increased seminal emissions (Bazzett et al., 1991). A D_1 agonist (dihydroxyphenyl-tetrahydrothienopyridine, THP) produced effects opposite those of the high dose of quinelorane: increased erections but fewer seminal emissions (Hull et al., 1992). Therefore, small increases in dopamine may disinhibit reflexes via D_2-like receptors; larger increases may facilitate parasympathetically mediated erections and inhibit sympathetically mediated seminal emission (i.e., prevent "premature ejaculation") via D_1-like receptors; and large increases may shift autonomic balance to favor seminal emission and ejaculation via a member of the D_2 receptor family (see Fig. 3). This interpretation is consistent with data on reflexes using high and low doses of the classic dopamine agonist apomorphine, together with D_1 and D_2 antagonists (Hull et al., 1992) and with effects on copulation of selective D_1 and D_2 agonists and antagonists (Hull et al., 1989). It is not clear whether the differential effects of low and high doses of D_2-like agonists are mediated by different members of the D_2 receptor family, with different affinities or efficacies, or by different groups of neurons that have different thresholds for activation.

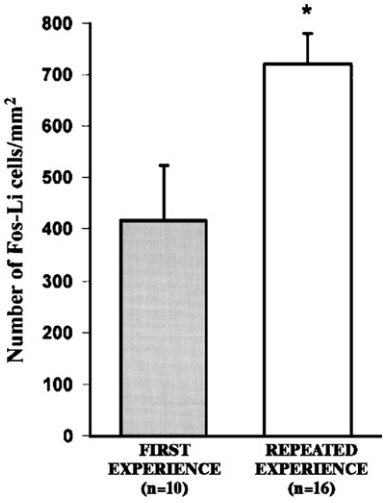

Fig. 2 – Male rats with previous sexual experience displayed greater Fos immunoreactivity in the medial preoptic nucleus (MPN), compared with those receiving their first sexual experience. All animals copulated to one ejaculation; however, experienced males had shorter mount, intromission, and ejaculation latencies and required fewer intromissions to elicit ejaculation. Values are expressed as mean ± SEM, *$p<0.02$. (Figure is from Lumley and Hull, 1999, with permission).

Extracellular dopamine in the MPOA rises as soon as a male rat detects the presence of an estrous female and remains high during copulation (Hull et al., 1995) (see Fig. 4). This dopamine response depends on the recent presence of gonadal hormones. One week after castration, two-thirds of the males showed a dopamine increase and copulated; the remaining third of the one-week castrates, and all of the two-week castrates, failed to show a dopamine increase and failed to copulate (Hull et al., 1995). There was a perfect correlation between a female-stimulated dopamine increase and the ability to copulate. Thus, recent, but not concurrent, testosterone was necessary for both the dopamine response and mating. The dopamine deficit in castrates is a chronic one, not just a loss of the acute response to the female as castrates also had lower extracellular dopamine during basal conditions, compared to gonadally intact males (Du et al., 1998). However, intracellular (stored) dopamine was actually higher in the castrates, and amphetamine induced a greater release of dopamine in castrates than in intact males. Therefore, castrates had abundant dopamine stored in tissue; their deficit was related to release, not synthesis, of dopamine.

4. Hormonal regulation of MPOA dopamine release

Testosterone is primarily a prohormone, which is either aromatized to estradiol or reduced to dihydrotestosterone in target tissues (reviewed in Hull et al., 2002). Estradiol-alone maintained normal basal levels of MPOA dopamine in castrated rats, but not the female-stimulated dopamine increase (Putnam et al., 2003) (see Fig. 5). Furthermore, all estradiol-treated castrates intromitted, but none ejaculated within the 30-min test period. Therefore, normal basal dopamine levels, maintained by estradiol, appeared to be sufficient for intromitting, but not for mating to ejaculation. Dihydrotestosterone-alone maintained neither basal levels nor female-stimulated dopamine increases, and no animal in that group copulated. However, the combination of estradiol plus dihydrotestosterone fully restored copulation, similarly to testosterone itself. There were numerous correlations between dopamine increases and copulatory measures. These data suggest that normal basal levels of dopamine in the MPOA are sufficient for at least some copulatory behavior, but that a female-stimulated increase, which requires androgen as well as estrogen, is required for efficient mating. These data are consistent with the microinjection results, which suggest that low to moderate levels of dopamine are sufficient to disinhibit genital reflexes and to stimulate parasympathetically mediated erection, but that higher levels of dopamine are required to elicit ejaculation, via D_2-like receptors.

Hormonal effects on intracellular (stored) dopamine are essentially opposite those on extracellular dopamine. As in

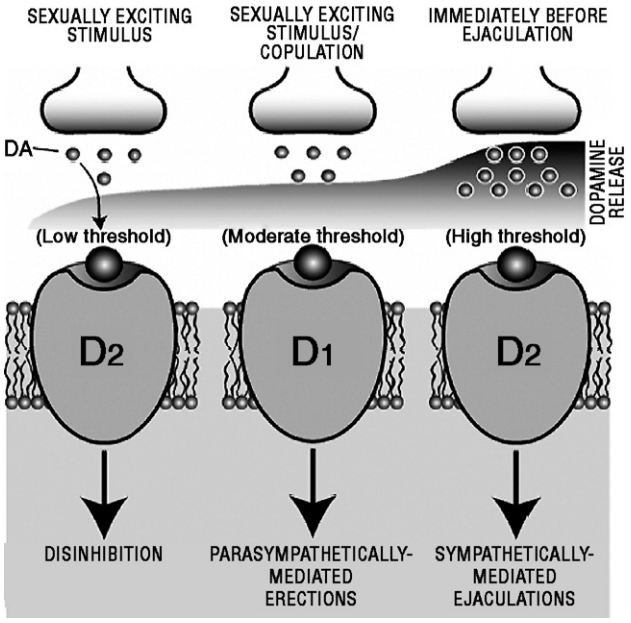

Fig. 3 – Model showing possible effects of D_1 versus D_2 stimulation in the MPOA, as a result of a sexually exciting stimulus and/or sexual activity (reviewed in Dominguez and Hull, 2005). In this model, a low-threshold mechanism mediated by a member of the D_2 receptor family disinhibits genital reflexes. A moderate threshold mechanism facilitates penile erections, after activation of D_1 receptors. A high threshold mechanism, activated by stimulation of a member of the D_2 receptor family, facilitates seminal emission/ejaculation and inhibits erections. These mechanisms may be activated successively by increasing levels or longer duration of DA activity. It is not clear whether the initial disinhibition and the triggering of ejaculation are mediated by different members of the D_2 receptor family or by different groups of neurons with different thresholds of activation. (Figure is from Dominguez and Hull, 2005, with permission).

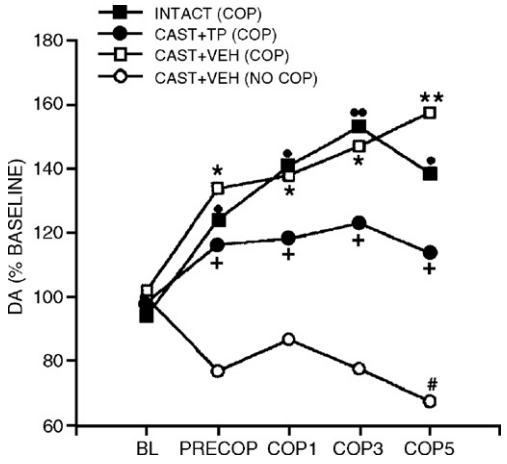

Fig. 4 – Levels of extracellular DA in the MPOA of male rats during baseline (BL), a precopulatory exposure to an estrous female (PRECOP), and three 6-min copulation samples (COP). Gonadally intact male rats showed an increase in extracellular DA during precopulatory exposure to an inaccessible estrous female, and all intact males copulated; males castrated 2 weeks before showed no DA release in response to the female, and none copulated. Values are expressed as mean ± SEM, *$p<0.05$, compared to final baseline for intact males or for one-week vehicle-treated castrates that copulated; **$p<0.01$, compared to final baseline for intact males or for one-week vehicle-treated castrates that copulated; +$p<0.05$, compared to baseline for testosterone-treated castrates; #$p<0.05$, compared to final baseline for vehicle-treated castrates that failed to copulate. (Figure is from Hull et al., 1995, with permission).

the Du et al. (1998) study, oil-treated castrates had the highest tissue (intracellular) dopamine followed by dihydrotestosterone-treated animals (Putnam et al., 2005) (see Fig. 6). Estradiol-alone maintained the lowest tissue dopamine, with estradiol + dihydrotestosterone and testosterone maintaining intermediate dopamine levels. Castrates treated with dihydrotestosterone-alone or oil showed little copulation, although somewhat more than in the Putnam et al., 2003 study, whereas testosterone and estradiol + dihydrotestosterone treatments maintained normal or near-normal mating, and estradiol-alone maintained intermediate mating ability. Therefore, animals with higher extracellular and lower intracellular dopamine levels had better copulatory ability. Again, castrates could readily synthesize dopamine but could not release their abundant intracellular stores.

5. The role of nitric oxide in dopamine release in the MPOA and the effects of experience

A major stimulus for MPOA dopamine release is the gaseous messenger molecule nitric oxide (NO). Reverse dialysis of the NO precursor L-arginine increased extracellular dopamine, and the NO synthase (NOS) inhibitor L-NMMA blocked that increase and decreased basal levels when administered alone (Lorrain and Hull, 1993). Reverse dialysis of a different NOS inhibitor, L-NAME, blocked the copulation-induced dopamine increase (Lorrain et al., 1996). Therefore, both basal and female-stimulated dopamine release is regulated by NO. Furthermore, neuronal NOS (nNOS) is hormonally regulated. Castration decreased nNOS immunoreactivity (ir), compared to both intact males and testosterone-treated castrates (Du and Hull, 1999). Tyrosine hydroxylase, the rate-limiting enzyme for dopamine synthesis, was not affected, again supporting the idea that dopamine synthesis is normal in castrates and that their deficit in extracellular dopamine is due to decreased release. Estradiol was the main factor maintaining nNOS-ir in the MPOA, with dihydrotestosterone being no more effective than oil, and estradiol + dihydrotestosterone or testosterone being comparable to estradiol-alone (Putnam et al., 2005). There was co-localization of nNOS-ir with both androgen receptor-ir and estrogen receptor-alpha-ir in several subareas of the MPOA, providing a means for hormonal regulation of its activity (Sato et al., 2005). In summary, estradiol up-regulates nNOS in the MPOA, with the resulting production of NO maintaining normal basal dopamine release and at least some copulation. The addition of androgen is required for the female-stimulated increase in dopamine and for efficient copulation. Androgen may work primarily on genital structures and genitosensory processing areas (Lugg et al., 1995; Meisel et al., 1984) and on

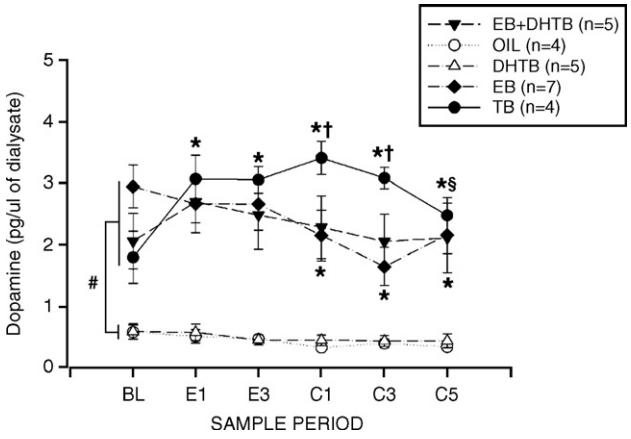

Fig. 5 – Temporal changes in dialysate concentrations of dopamine during copulation in the MPOA of male rat castrates treated with various hormone regimens (EB, estradiol benzoate; DHTB, dihydrotestosterone benzoate; Oil, vehicle; TP, testosterone propionate). Samples include baseline (BL), two precopulatory 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 odd-numbered samples are depicted, for greater clarity.) *$p<0.05$ different from respective baseline (within-group); §$p<0.05$ different from respective COP3 (within-group); †$p<0.05$ different from EB and EB + DHTB groups (between-group); #$p<0.05$ difference between oil and DHTB groups and the three other groups for every sample period (between-group). Values are expressed as mean ± SEM. (Figure is from Putnam et al., 2003, with permission).

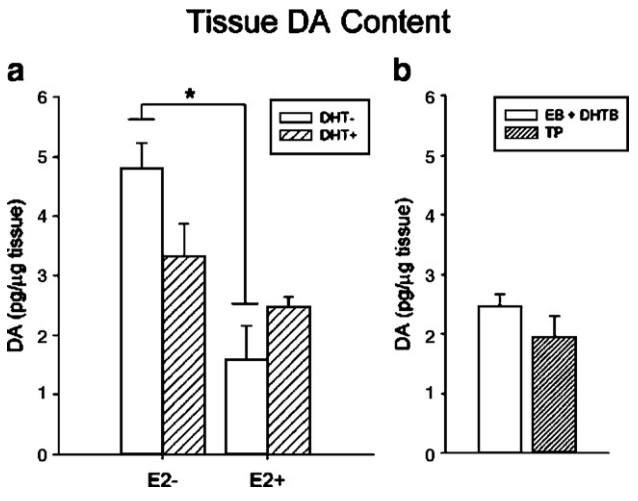

Fig. 6 – Tissue content of DA in the MPOA of castrates treated with various hormone regimens. (a) Comparison among animals without or with estradiol benzoate (EB) (E2– vs. E2+) and/or dihydrotestosterone benzoate (DHTB) (DHT– vs. DHT+). Castrates with only EB (E2+/DHT–) had lower levels of tissue dopamine content, compared to castrates treated only with oil (E2–/DHT–). The difference between castrates with only DHTB (E2–/DHT+) and those with EB + DHTB (E2+/DHT+) was not statistically significant. (b) Comparison between EB + DHTB and TP-treated animals. This difference was not statistically significant. Values are expressed as mean ± SEM, *$p<0.05$. (Figure is from Putnam et al., 2005, with permission).

chemosensory processing areas, including the MeA and BNST (Simerly et al., 1990; Wood and Newman, 1993).

NO in the MPOA also affects copulation. Reverse dialysis of the NO precursor L-arginine facilitated mounting, and similar administration of the NOS inhibitor L-NMMA inhibited mounting (Sato et al., 1998). Microinjections of L-NAME into the MPOA completely blocked copulation in sexually naive males and impaired the behavior in experienced males (Lagoda et al., 2004). It also blocked the facilitative effects of seven non-copulatory exposures of sexually naive males to an estrous female, seen in saline-treated naive males, compared to naive males not pre-exposed to a female (see Fig. 7). Specifically, saline-treated female-exposed males had more mounts, intromissions, and ejaculations than either L-NAME-treated or non-exposed control males.

A major stimulus for the activation of NOS is glutamate, acting via NMDA receptors to admit Ca^{2+}, which activates calmodulin, which in turn activates NOS (reviewed in Aoki et al., 1998; Garthwaite and Boulton, 1995). Indeed, microinjections of the glutamate NMDA receptor antagonist MK-801 produced behavioral effects similar to those observed with the NOS inhibitor L-NAME (Vigdorchik et al., 2003). MK-801 impaired copulation in both naive and experienced males and blocked the effects of seven pre-exposures to estrous females. Therefore, either inhibiting NOS directly with L-NAME (Lagoda et al., 2004) or blocking NMDA receptors that typically activate NOS (Vigdorchik et al., 2003) impaired copulation and prevented the facilitative effects of pre-exposures to an estrous female.

If NO mediates at least some of the facilitative effects of experience, the next question is whether nNOS, itself, is up-regulated by sexual experience. Indeed, nNOS protein was increased in Western blots from males with previous experience, whether or not they mated on the day of sacrifice (Fig. 8A, Dominguez et al., in press). Similarly, the number of nNOS-ir cells in the medial preoptic nucleus (MPN) was higher in males that had previous sexual experience, whether or not they mated on the day of sacrifice (Fig. 8B, Dominguez et al., in press). Therefore, one mechanism for enhanced copulation in sexually experienced males is increased expression of nNOS, which would result in greater production of NO, which would in turn increase MPOA dopamine release, and, consequently, enhance copulation.

The most common second messenger of NO is cGMP (reviewed in West et al., 2002), although NO's effects are sometimes mediated by ADP-ribosylation of presynaptic proteins (Kleppisch et al., 1999; Schuman et al., 1994) or S-nitrosylation of channel proteins (reviewed in Ahern et al., 2002). The effects of NO on MPOA dopamine levels and on copulation are mediated primarily via activation of soluble guanylyl cyclase and production of cGMP. Reverse dialysis of a membrane-permeable analog of cGMP (8-Br-cGMP) increased extracellular dopamine, whereas the guanylyl cyclase inhibitor ODQ decreased dopamine levels (Sato and Hull, 2006). ODQ also blocked the dopamine increase elicited by the NO donor sodium nitroprusside (SNP), showing that NO needed cGMP to mediate its effects. However, the NOS inhibitor L-NMMA did not block the increase elicited by 8-Br-cGMP (see Fig. 9);

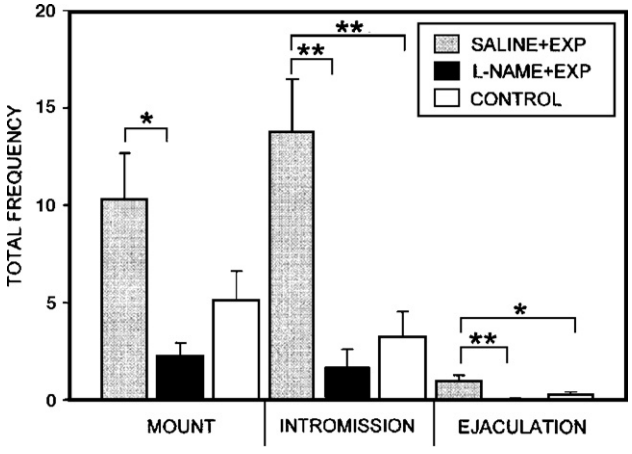

Fig. 7 – Mean total mounts, intromissions, and ejaculations in sexually naive rats treated with the nitric oxide synthase inhibitor L-NAME (100 µg/µl) or saline before each of seven non-copulatory exposures to a receptive female and tested drug free on Day 8 and in untreated naive males not pre-exposed to a female. Open bars indicate naive rats that did not receive pre-exposure, gray bars indicate rats that received a saline microinjection before each exposure, and black bars indicate naive rats that received an L-NAME microinjection before each exposure. Exposure to females consisted of placing the female in a wire mesh cage over the male's home cage for 30 min. Values are expressed as mean ± SEM, *$p<0.05$, **$p<0.01$. (Figure is from Lagoda et al., 2004, with permission).

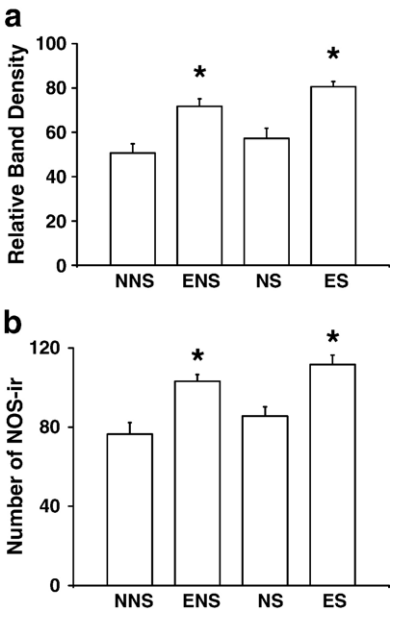

Fig. 8 – (a) Protein concentration for nNOS in the MPOA of male rats in the following groups: sexually naive rats that did (NS, naive/sex) or did not (NNS, naive/no-sex) mate on the day of testing and sexually experienced rats that did (ES, experienced/sex) or did not (ENS, experienced/no-sex) mate on day of testing. (b) Average number of nNOS-immunoreactive cells in the MPOA of male rats undergoing these experience and mating conditions. Values are expressed as mean ± SEM, $^{*}p<0.05$, compared to both NS and NNS groups. (Figures are from Dominguez et al., in press, with permission).

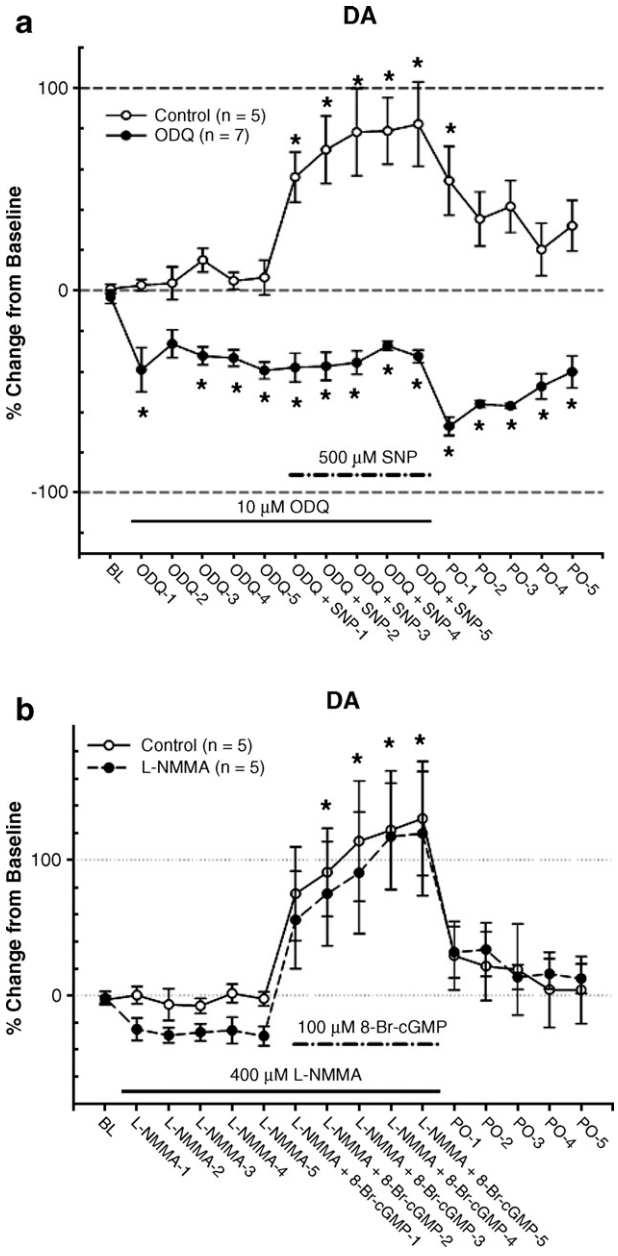

Fig. 9 – (a) Effects of pre-treatment with the guanylyl cyclase inhibitor ODQ on increases in MPOA dopamine elicited by the nitric oxide donor sodium nitroprusside SNP. Data for control animals (without ODQ pretreatment) are shown as open circles, and the data for the ODQ animals are shown as closed circles. ODQ blocked the effects of SNP, showing that cGMP is "downstream" from nitric oxide. (b) Effects of pre-treatment with the nitric oxide synthase inhibitor L-NMMA on increases in MPOA dopamine elicited by the cGMP analog 8-Br-cGMP. Measures for control animals (without L-NMMA-pretreatment) are shown as open circles, and data for L-NMMA animals are shown as closed circles (BL is baseline). L-NMMA failed to block the effects of 8-Br-cGMP, showing that nitric oxide is "upstream" of cGMP. Values are expressed as mean ± SEM, $^{*}p<0.05$. (Figure is from Sato and Hull, 2006, with permission).

therefore, cGMP is "downstream" from NO and thus does not need NO to mediate its effects. It is not clear how the cGMP effect is mediated; however, in the striatum, cGMP exerts a tonic excitatory influence on medium spiny neurons (West and Galloway, 2004); perhaps a similar facilitative effect occurs in dopamine- or glutamate-containing neurons in the MPOA. In addition to increasing dopamine release, 8-Br-cGMP facilitated copulation, increasing the number of ejaculations per 30-min test and decreasing the time and intromissions required to trigger the second and third ejaculations. Thus, the cGMP analog decreased the threshold for ejaculation and thereby increased the number of ejaculations per 30-min test. Conversely, the guanylyl cyclase inhibitor ODQ decreased the total numbers of intromissions and ejaculations during the test. Therefore, NO, acting primarily via cGMP, increases MPOA dopamine release and increases ejaculations.

6. The role of glutamate in dopamine release and sexual experience

Since NO regulates dopamine levels in the MPOA and since a major activator of NOS is glutamate acting on NMDA receptors, we verified that, indeed, reverse dialysis of glutamate into the MPOA increases dopamine levels (Dominguez et al., 2004). A small increase elicited by 1 mM glutamate in the

dialysate was not statistically significant; however, 10 mM glutamate elicited a significant 200% dopamine increase. One question is whether the dopamine increase elicited by 10 mM glutamate resulted from excitotoxic damage. This is unlikely because dopamine levels fell towards baseline as soon as the glutamate was removed. In addition, *in vitro* probe recovery for both dopamine and glutamate was about 20%, so only about one fifth of the concentration of glutamate in the dialysate reached the tissue (~2 mM). In contrast to these data, dopamine was elevated for at least 24 h following excitotoxic damage to the striatum (Shimizu et al., 2003). In addition, the MPOA appears to be unusually resistant to excitotoxic damage since microinjection of 1 μl of 120 mM NMDA into the MPOA resulted in a loss of no more than 10% of Nissl-stained neurons in male hamsters (Ebling et al., 1998). In comparison, 1 μl of 12 mM NMDA (a 10-fold lower dose) into the striatum or cortex caused a loss of more than 90% of neurons in those areas. Therefore, the dopamine release elicited by 10 mM glutamate in the dialysate was probably not a result of excitotoxic damage.

Although glutamate increased extracellular dopamine, levels of the dopamine metabolites DOPAC and HVA were actually decreased (Dominguez et al., 2004). Dopamine must be transported back into the terminal in order to be metabolized to DOPAC by monoamine oxidase; subsequently, DOPAC can diffuse outside the terminal and be converted to HVA by catechol-O-methyl transferase (reviewed in Cooper et al., 2003). Therefore, the increase in dopamine, together with decreases in DOPAC and HVA, suggests that the dopamine transporter had been inhibited. As will be discussed below, NO has been reported to inhibit the dopamine transporter.

Both basal and glutamate-stimulated dopamine levels are regulated by NO. Reverse dialysis of the NOS inhibitor L-NAME alone decreased basal levels of dopamine and blocked the increase elicited by glutamate in control animals; L-NAME also blocked the decrease in the metabolites. The inactive isomer D-NAME had no effect. Therefore, glutamate increased extracellular dopamine largely by increasing production of NO. There are at least two possible ways in which glutamate could increase extracellular dopamine. First, it could directly induce exocytosis at dopamine terminals or increase firing of dopamine cell bodies. Second, it could bind to NMDA receptors, allowing Ca^{2+} influx and consequent activation of calmodulin and NOS (see Fig. 10). The increased production of NO could elicit exocytosis of dopamine and inhibit the dopamine transporter (reviewed in West et al., 2002; Kiss and Vizi, 2001). The ability of L-NAME to block the glutamate-induced increase in dopamine suggests that the second, NO-mediated, mechanism is the predominant one in the MPOA. Furthermore, the glutamate-induced decrease in dopamine metabolites suggests that the transporter was, in fact, inhibited.

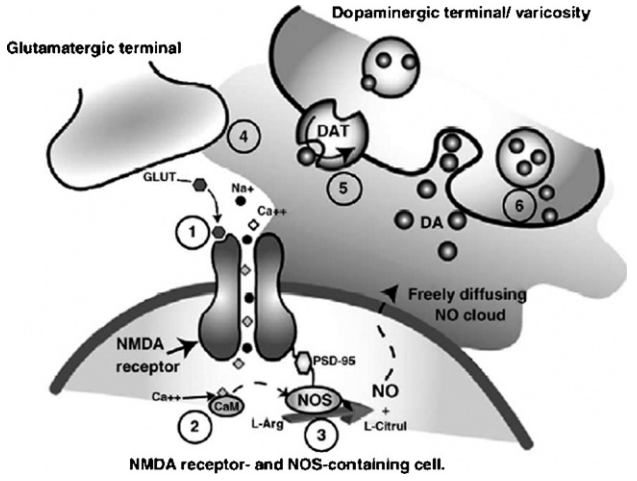

Fig. 10 – Model showing possible interactions between glutamate, nitric oxide (NO), and dopamine in the MPOA. (1) Glutamate (GLUT; gray hexagon) activates NMDA receptors, which opens calcium channels. (2) The resultant increase in intracellular calcium (gray diamonds) then activates calmodulin (CaM), (3) which in turn activates the enzyme NO synthase (NOS); this leads to an immediate production of NO. NOS links to the carboxy-terminal tail of the NMDA receptor, via a PSD-95 protein–protein interaction domain. Once synthesized, NO freely diffuses from cell to cell, (4) where it can alter activity in the presynaptic neurons. (5) Additionally, in dopamine-producing neurons, NO has been shown to inhibit the dopamine transporter (DAT) (6) and increase calcium-dependent and/or calcium-independent vesicular release. Therefore, increased NO in the MPOA, after glutamate release, would increase levels of extracellular dopamine and prolong the presence of dopamine in the synapse. (Figure is from Dominguez and Hull, 2005, with permission).

7. Where does the glutamate stimulus originate?

The neural stimulus for female-elicited dopamine release arrives at the MPOA from the MeA, either directly or via the BNST. Large excitotoxic lesions of the amygdala severely impaired copulatory ability, which was restored by microinjections of the dopamine agonist apomorphine into the MPOA (Dominguez et al., 2001), suggesting that a major way in which the amygdala facilitates copulation is by increasing MPOA dopamine release. Furthermore, smaller radio-frequency lesions of the MeA did not affect basal dopamine levels, but did block the female-elicited increase; males with these MeA lesions had half the number of ejaculations as did sham-lesion animals. As with estradiol-treated castrates, maintenance of normal basal levels of dopamine in the MPOA was sufficient for at least some copulation, but the additional female-stimulated increase was necessary for optimal copulation. In male hamsters, too, chemosensory input is necessary for MPOA dopamine release; ipsilateral, but not contralateral, olfactory bulbectomy blocked mating-induced dopamine release in the MPOA (Triemstra et al., 2005). Finally, chemical stimulation of the male rat amygdala elicited an increase in MPOA dopamine comparable to that induced by a female (Dominguez and Hull, 2001). Therefore, sensory input from the MeA is necessary for the female-stimulated dopamine increase in the MPOA and for efficient copulation. Although the initial increase in dopamine is elicited primarily by chemosensory stimuli, additional genito-sensory input during copulation may contribute to the

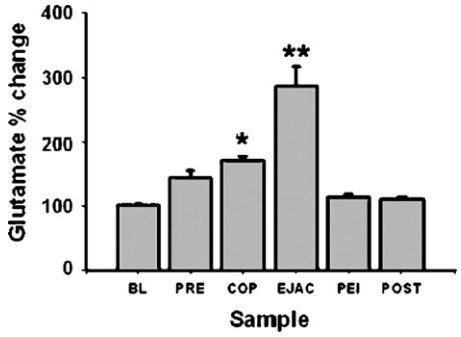

Fig. 11 – Changes in extracellular MPOA glutamate during copulation. Samples analyzed included baseline (BL), precopulation (PRE: female placed above the male's cage), copulation (COP: only mounts and intromissions), ejaculation (EJAC), post-ejaculatory interval (PEI), and post-copulation (POST) samples. Samples were collected at 2 min intervals. Values are expressed as mean ± SEM. $^{*}p<0.05$; $^{**}p<0.001$. (Figure is from Dominguez et al., 2006, with permission).

prolonged increase. Therefore, projections from the MeA, either direct or via the BNST, may increase MPOA dopamine release in response to both chemosensory and genitosensory stimuli.

The means by which the amygdala elicits dopamine release in the MPOA was not clear since there are no dopamine-containing neurons in the amygdala of rats (Björklund and Lindvall, 1984). However, glutamatergic input could stimulate the dopamine release. A few axons in the MPOA from the MeA, and more from the BNST, contained the vesicular glutamate transporter and therefore appeared to be glutamatergic (Dominguez et al., 2003). Furthermore, extracellular glutamate in the MPOA increased during copulation and peaked at 300% of baseline in the two-minute sample during which the male ejaculated (Dominguez et al., 2006) (see Fig. 11). Glutamate levels fell to baseline during the post-ejaculatory interval, and the amount of that decrease was positively correlated with the duration of the post-ejaculatory interval before resuming copulation. Finally, reverse dialysis of a cocktail of neuronal and glial glutamate reuptake inhibitors increased extracellular glutamate levels, as expected, and also decreased ejaculation latencies and post-ejaculatory intervals and increased the numbers of ejaculations per 30-minute test (Dominguez et al., 2006). Previous research has shown that glutamate micro-injections into the MPOA of anesthetized male rats increased erectile responses (Giuliano et al., 1997) and elicited the urethrogenital reflex, a model of orgasm in humans (Marson and McKenna, 1994). Therefore, extracellular glutamate increases in the MPOA during copulation and facilitates several components of male sexual behavior. As noted above, it may also lead to the production of NO, which contributes to the facilitative effects of sexual experience.

8. Summary

In summary, chemosensory information is processed by the main and accessory olfactory systems and relayed to the MeA, which in turn processes and relays it to the MPOA, both directly and via the BNST. Genitosensory information is also relayed to the MeA and the MPOA from the CTF. At least some of the input from the MeA and BNST is glutamatergic. Glutamate may increase MPOA dopamine release directly by increasing dopamine cell firing and/or exocytosis from terminals. It may also activate NMDA receptors, thereby allowing Ca^{2+} to enter NOS-containing cells and activate calmodulin, which in turn activates NOS. The resultant NO production may directly induce dopamine exocytosis and also inhibit the dopamine transporter, which allows released dopamine to remain in the synapse longer. Small increases in dopamine disinhibit genital reflexes via D_2-like receptors; moderate increases stimulate erection and early stages of copulation via D_1-like receptors; and large increases facilitate seminal emission and ejaculation via a member of the D_2 receptor family. Glutamate may also stimulate reflexes and copulation directly. Hormones regulate dopamine release, at least in part, by up-regulating nNOS in the MPOA. Previous sexual experience also increases the amount of nNOS in the MPOA, thereby increasing production of NO and consequently dopamine (and possibly glutamate) release. The increased dopamine and glutamate may explain the finding of greater ejaculation-induced Fos-ir in the MPOA of sexually experienced males, in spite of their having had fewer intromissions, compared to sexually naive males. Finally, the establishment of sexual experience effects requires activation of both NMDA receptors and NOS.

Acknowledgment

Supported by NIH grants K02 MH 01714 and R01 MH 40826 to E.M.H.

REFERENCES

Ahern, G.P., Klyachko, V.A., Jackson, M.B., 2002. cGMP and S-nitrosylation: two routes for modulation of neuronal excitability by NO. Trends Neurosci. 25, 510–517.

Aoki, C., Bredt, D.S., Fenstemaker, S., Lubin, M., 1998. The subcellular distribution of nitric oxide synthase relative to the NR1 subunit of NMDA receptors in the cerebral cortex. In: Mize, R.R., Dawson, T.M., Dawson, V.L., Friedlander, M.J. (Eds.), Progress in Brain Research, Vol. 118, Nitric Oxide in Brain Development Plasticity, and Disease. Elsevier, Amsterdam, pp. 83–97.

Barfield, R.J., Thomas, D.A., 1986. The role of ultrasonic vocalizations in the regulation of reproduction in rats. Ann. N. Y. Acad. Sci. 474, 33–43.

Baum, M.J., Everitt, B.J., 1992. Increased expression of c-fos in the medial preoptic area after mating in male rats: role of afferent inputs from the medial amygdala and midbrain central tegmental field. Neuroscience 50, 627–646.

Bazzett, T.J., Eaton, R.C., Thompson, J.T., Markowski, V.P., Lumley, L.A., Hull, E.M., 1991. Dose dependent D2 effects on genital reflexes after MPOA injections of quinelorane and apomorphine. Life Sci. 48, 2309–2315.

Björklund, A., Lindvall, O., 1984. Dopamine-containing systems in the CNS. In: Björklund, A., Hökfelt, T. (Eds.), Handbook of Chemical Neuroanatomy. Classical Transmitters in the CNS, Vol. 2: Part I. Elsevier, Amsterdam, pp. 55–122.

Claro, F., Segovia, S., Guilamon, A., Del Abril, A., 1995. Lesions in the medial posterior region of the BST impair sexual behavior in sexually experienced and inexperienced male rats. Brain Res. Bull. 36, 1–10.
Coolen, L.M., Allard, J., Truitt, W.A., McKenna, K.E., 2004. Central regulation of ejaculation. Physiol. Behav. 83, 203–215.
Cooper, J.R., Bloom, F.E., Roth, R.H., 2003. The Biochemical Basis of Neuropharmacology, 8th ed. Oxford Univ. Press, New York, pp. 231–236.
de Jonge, F.H., Louwerse, A.L., Ooms, M.P., Evers, P., Endert, E., van de Poll, N.E., 1989. Lesions of the SDN-POA inhibit sexual behavior of male Wistar rats. Brain Res. Bull. 23, 483–492.
Dewsbury, D.A., 1969. Copulatory behavior of rats (*Rattus norvegicus*) as a function of prior copulatory experience. Anim. Behav. 17, 217–223.
Dominguez, J.M., Hull, E.M., 2001. Stimulation of the medial amygdala enhances medial preoptic dopamine release: implications for male rat sexual behavior. Brain Res. 917, 225–229.
Dominguez, J.M., Hull, E.M., 2005. Dopamine, the medial preoptic area, and male sexual behavior. Physiol Behav. 86, 356–368.
Dominguez, J., Riolo, J.V., Xu, Z., Hull, E.M., 2001. Regulation by the medial amygdala of copulation and medial preoptic dopamine release. J. Neurosci. 21, 349–355.
Dominguez, J.M., Balfour, M.E., Coolen, L.M., 2003. Copulation-induced activation of NMDA receptor containing neurons in the medial preoptic nucleus. Horm Behav 44, 46.
Dominguez, J.M., Muschamp, J.W., Schmich, J.M., Hull, E.M., 2004. Nitric oxide mediates glutamate-evoked dopamine release in the medial preoptic area. Neuroscience 125, 203–210.
Dominguez, J.M., Gil, M., Hull, E.M., 2006. Preoptic glutamate facilitates male sexual behavior. J Neurosci. 26, 1699–1703.
Dominguez, J.M., Brann, J.H., Gil, M., Hull, E.M., in press. Sexual experience increases nitric oxide synthase in the medial preoptic area of male rats. Behavioral Neuroscience.
Du, J., Hull, E.M., 1999. Effects of testosterone on neuronal nitric oxide synthase and tyrosine hydroxylase. Brain Res. 836, 90–98.
Du, J., Lorrain, D.S., Hull, E.M., 1998. Castration decreases extracellular, but increases intracellular, dopamine in medial preoptic area of male rats. Brain Res. 782, 11–17.
Ebling, F.J.P., Cronin, A.S., Hastings, M.H., 1998. Resistance of gonadotropin-releasing hormone neurons to glutamatergic neurotoxicity. Brain Res Bull. 47, 575–584.
Garthwaite, J., Boulton, C.L., 1995. Nitric oxide signaling in the central nervous system. Annu. Rev. Physiol. 57, 683–706.
Giuliano, F., Bernabé, J., Brown, K., Droupy, S., Benoit, G., Rampin, O., 1997. Erectile response to hypothalamic stimulation in rats: role of peripheral nerves. Am. J. Physiol. 273, R1990–R1997.
Hull, E.M., Warner, R.K., Bazzett, T.J., Eaton, R.C., Thompson, J.T., Scaletta, L.L., 1989. D2/D1 ratio in the medial preoptic area affects copulation of male rats. J. Pharmacol. Exp. Ther. 251, 422–427.
Hull, E.M., Eaton, R.C., Markowski, V.P., Moses, J., Lumley, L.A., Loucks, J.A., 1992. Opposite influence of medial preoptic Dl and D2 receptors on genital reflexes: implications for copulation. Life Sci. 51, 1705–1713.
Hull, E.M., Du, J., Lorrain, D.S., Matuszewich, L., 1995. Extracellular dopamine in the medial preoptic area: implications for sexual motivation and hormonal control of copulation. J. Neurosci. 15, 7465–7471.
Hull, E.M., Meisel, R.L., Sachs, B.D., 2002. Male sexual behavior. In: Pfaff, D.W., Arnold, A.P., Etgen, A.M., Fahrbach, S.E., Rubin, R.T. (Eds.), Hormones, Brain and Behavior. Academic Press, pp. 3–137.
Hull, E.M., Wood, R.I., McKenna, K.E., 2006. The neurobiology of male sexual behavior, In: Neill, J., Pfaff, Donald (Eds.), The Physiology of Reproduction, 3rd ed. Elsevier Press, Amsterdam, pp. 1729–1824.
Kiss, J.P., Vizi, E.S., 2001. Nitric oxide: a novel link between synaptic and nonsynaptic transmission. Trends Neurosci. 24, 211–215.
Kleppisch, T., Pfeifer, A., Klatt, P., Ruth, P., Montkowski, A., Fassler, R., Hofmann, F., 1999. Long-term potentiation in the hippocampal CA1 region of mice lacking cGMP-dependent kinases is normal and susceptible to inhibition of nitric oxide synthase. J. Neurosci. 19, 48–55.
Kondo, Y., 1992. Lesions of the medial amygdala produce severe impairment of copulatory behavior in sexually inexperienced male rats. Physiol. Behav. 51, 939–943.
Lagoda, G., Muschamp, J.M., Vigdorchik, A., Hull, E.M., 2004. A nitric oxide synthase inhibitor in the medial preoptic area inhibits copulation and stimulus sensitization in male rats. Behav. Neurosci. 118, 1317–1323.
Larsson, K., 1959. Experience and maturation in the development of sexual behaviour in the male puberty rat. Behaviour 14, 101–107.
Lopez, H.H., Olster, D.H., Ettenberg, A., 1999. Sexual motivation in the male rat: the role of primary incentives and copulatory experience. Horm. Behav. 36, 176–185.
Lorrain, D.S., Hull, E.M., 1993. Nitric oxide increases dopamine and serotonin release in the medial preoptic area. NeuroReport 5, 87–89.
Lorrain, D.S., Matuszewich, L., Howard, R.V., Du, J., Hull, E.M., 1996. Nitric oxide promotes medial preoptic dopamine release during male rat copulation. NeuroReport 8, 31–34.
Lugg, J.A., Rajfer, J., Gonzalez-Cadavid, N.F., 1995. Dihydrotestosterone is the active androgen in the maintenance of nitric oxide-mediated penile erection in the rat. Endocrinology 136, 1495–1501.
Lumley, L.A., Hull, E.M., 1999. Effects of a Dl antagonist and of sexual experience on copulation-induced Fos-like immunoreactivity in the medial preoptic nucleus. Brain Res. 829, 55–68.
Marson, L., McKenna, K.E., 1994. Stimulation of the hypothalamus initiates the urethrogenital reflex in male rats. Brain Res. 638, 103–108.
Meisel, R.L., O'Hanlon, J.K., Sachs, B.D., 1984. Differential maintenance of penile responses and copulatory behavior by gonadal hormones in castrated male rats. Horm. Behav. 18, 56–64.
Pfaus, J.G., Wilkins, M.F., 1995. A novel environment disrupts copulation in sexually naive but not experienced male rats: reversal with naloxone. Physiol. Behav. 57, 1045–1049.
Powell, W.S., Dominguez, J.M., Hull, E.M., 2003. An NMDA antagonist impairs copulation and the experience-induced enhancement of male sexual behavior in the rat. Behav. Neurosci. 117, 69–75.
Putnam, S.K., Sato, S., Hull, E.M., 2003. Hormonal maintenance of copulation in castrates: association with extracellular dopamine in MPOA. Horm. Behav. 44, 419–426.
Putnam, S.K., Sato, S., Riolo, J.V., Hull, E.M., 2005. Effects of testosterone metabolites on copulation, medial preoptic dopamine, and NOS-immunoreactivity in castrated male rats. Horm. Behav. 47, 513–522.
Saito, T.R., Moltz, H., 1986. Copulatory behavior of sexually naive and sexually experienced male rats following removal of the vomeronasal organ. Physiol. Behav. 37, 507–510.
Sato, S.M., Hull, E.M., 2006. The nitric oxide-guanosine 3′,5′-cyclic monophosphate pathway regulates dopamine efflux in the medial preoptic area and copulation in male rats. Neuroscience 139, 417–428.
Sato, Y., Horita, H., Kurohata, T., Adachi, H., Tsukamoto, T., 1998. Effect of the nitric oxide level in the medial preoptic area on male copulatory behavior in rats. Am. J. Physiol. 274, R243–R247.
Sato, S., Braham, C.S., Putnam, S.K., Hull, E.M., 2005. Neuronal nitric oxide synthase and gonadal steroid interaction in the MPOA of male rats: co-localization and testosterone-induced

restoration of copulation and nNOS-immunoreactivity. Brain Res. 1043, 205–213.
Schuman, E.M., Meffert, M.K., Schulman, H., Madison, D.V., 1994. An ADP-ribosyltransferase as a potential target for nitric oxide action in hippocampal long-term potentiation. Proc. Natl. Acad. Sci. U. S. A. 91, 11958–11962.
Shimizu, K., Matsubara, K., Ohtaki, K., Fujimaru, S., Saito, O., Shiono, H., 2003. Paraquat induces long-lasting dopamine overflow through the excitotoxic pathway in the striatum of freely moving rats. Brain Res. 976, 243–252.
Simerly, R.B., 1995. Hormonal regulation of limbic and hypothalamic pathways. In: Micevych, P.E., Hammer Jr., R. (Eds.), Neurobiological Effects of Sex Steroid Hormones. Cambridge Univ. Press, Cambridge, pp. 85–116.
Simerly, R.B., Swanson, L.W., 1986. The organization of neural inputs to the medial preoptic nucleus of the rat. J. Comp. Neurol. 246, 312–342.
Simerly, R.B., Swanson, L.W., 1988. Projections of the medial preoptic nucleus: a *Phaseolis vulgaris* leucoagglutinin anterograde tract-tracing study in the rat. J. Comp. Neurol. 270, 209–242.
Simerly, R.B., Chang, C., Muramatsu, M., Swanson, L.W., 1990. Distribution of androgen and estrogen mRNA-containing cells in the rat brain: an in situ hybridization study. J. Comp. Neurol. 294, 76–95.
Triemstra, J.L., Nagatani, S., Wood, R.I., 2005. Chemosensory cues are essential for mating-induced dopamine release in MPOA of male Syrian hamsters. Neuropsychopharmacology 30, 1436–1442.
Vigdorchik, A., Lagoda, G., Hull, E.M., 2003. A glutamate receptor antagonist microinjected into the MPOA impairs male sexual behavior. Horm. Behav. 44, 82.
West, A.R., Galloway, M.P., 2004. The nitric oxide-guanylyl cyclase signaling pathway modulates membrane activity states and electrophysiological properties of striatal medium spiny neurons recorded *in vivo*. J. Neurosci. 24, 1924–1935.
West, A.R., Galloway, M.P., Grace, A.A., 2002. Regulation of striatal dopamine neurotransmission by nitric oxide: effector pathways and signaling mechanisms. Synapse 44, 227–245.
Wood, R.I., Newman, S.W., 1993. Mating activates androgen receptor-containing neurons in the chemosensory pathways of the male Syrian hamster brain. Brain Res. 614, 65–77.
Wood, R.I., Newman, S.W., 1995. Hormonal influence on neurons of the mating behavior pathway in male hamsters. In: Micevych, P.E., Hammer Jr., R.P. (Eds.), Neurobiological Effects of Sex Steroid Hormones. Cambridge Univ. Press, New York, pp. 3–39.