Behavioral Neuroscience
2004, Vol. 118, No. 6, 1317–1323

0735-7044/04/$12.00 DOI: 10.1037/0735-7044.118.6.1317

A Nitric Oxide Synthesis Inhibitor in the Medial Preoptic Area Inhibits Copulation and Stimulus Sensitization in Male Rats

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Dopamine in the medial preoptic area (MPOA) facilitates copulation in male rats, and nitric oxide (NO) regulates basal and female-stimulated MPOA dopamine release. Microinjection of L-nitro-arginine methyl ester (L-NAME, an NO synthesis inhibitor) into the MPOA blocked copulation in naive rats and impaired copulation in sexually experienced males. In other naive rats, L-NAME or saline was microinjected into the MPOA before each of 7 daily exposures to a receptive female placed over their cage. In a drug-free test on Day 8, copulation by L-NAME-treated rats was similar to that of unexposed controls and was impaired relative to saline-treated males. Therefore, NO in the MPOA is important for copulation and stimulus sensitization in male rats.

Nitric oxide (NO) may facilitate male sexual behavior in several ways. NO acts peripherally as a vasodilator, causing relaxation of the corpus cavernosum and thereby leading to erection (Ignarro et al., 1990; Kim, Azadzoi, Goldstein, & Saenz de Tejada, 1991). Systemic injections of the NO synthase (NOS) inhibitor L-nitro-arginine methyl ester (L-NAME) impaired copulation in sexually experienced male rats by reducing the numbers of intromissions and ejaculations (Benelli et al., 1995; Bialy, Beck, Abramczyk, Trzebski, & Przbylski, 1996; Hull et al., 1994). Systemically administered L-NAME also decreased the number of ex copula erections but increased the number of seminal emissions (Hull et al., 1994). In contrast, intracerebroventricular injections had no adverse effect on copulatory ability (Benelli et al., 1995). Therefore, peripheral vascular effects of systemically injected L-NAME may have mediated its inhibitory effects on copulation in sexually experienced male rats.

In sexually naive male rats, systemic injections of the same doses of L-NAME produced more severe impairment of copulation, and intracerebroventricular administration completely prevented ejaculation (Benelli et al., 1995). Thus, naive rats may be more vulnerable to the effects of NOS inhibitors, as L-NAME prevented ejaculation in naive rats but did not decrease the numbers of ejaculations in experienced rats.

The medial preoptic area (MPOA) is one of the most integral sites controlling sexual behavior in male mammals (reviewed in Hull, Meisel, & Sachs, 2002). NOS-containing neurons are located in this area (Vincent & Kimura, 1992). Sato, Horita, Kurohata, Adachi, and Tsukamoto (1998) reported that infusion of a different NOS inhibitor, N-monomethyl L-arginine (L-NMMA), through a microdialysis probe into the MPOA of sexually experienced male rats significantly reduced mount rate and the percentage of rats that ejaculated during a 10-min copulatory test but did not affect the intromission ratio (ratio of intromissions to mounts-plus-intromissions).

In order to test more fully the central effects of an NOS inhibitor on sexual behavior, we microinjected L-NAME or saline into the MPOA of sexually naive male rats and tested their copulatory ability for 30 min. The rats were then given sexual experience and tested again with L-NAME and saline in counterbalanced order. We hypothesized that L-NAME would decrease copulation in both naive and sexually experienced males.

Because naive rats appear to be more sensitive than experienced rats to the disruptive effects of central NOS inhibition (Benelli et al., 1995), and because nitric oxide plays an important role in experience-dependent plasticity (Hawkins, Son, & Arancio, 1998), we evaluated the effect of repeated MPOA microinjections of L-NAME on the subsequent copulatory ability of sexually naive males before each of seven noncopulatory exposures to an estrous female. Typically, preexposure to female odors facilitates later sexual performance in naive males (de Jonge, Oldenburger, Louwerse, & van de Poll, 1992; Powell, Dominguez, & Hull, 2003). However, we hypothesized that chronic L-NAME microinjections would block the normal facilitation in sexual performance and render the L-NAME-treated rats inferior copulators compared with saline-treated males and similar to naive rats that were never preexposed to a receptive female.

Method

Animals and Surgery

Fourteen Long Evans/Blue Spruce (Harlan, Indianapolis, IN; 300–350 g) male rats were used for acute microinjections (Experiment 1), and 42 rats were used for repeated microinjections preceding exposures to an inaccessible receptive female (Experiment 2). They were housed individually in large plastic cages. Both the male and female colony rooms were climate controlled on a 14:10-hr light–dark cycle with lights off at 11 a.m. and on at 9 p.m. Food and water were available ad libitum. Before intracranial surgery, rats were anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4 mg/kg) and then implanted with a 23-g stainless steel guide cannula ending 1 mm above the MPOA. Stereotaxic coordinates (from bregma, AP +2.1, ML +0.4, DV −6.3 from

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This research was supported by National Institutes of Mental Health Grants MH R01–40826 and MH K02–001714 to Elaine M. Hull.

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dura) used for the anterior MPOA were adapted from Pellegrino, Pellegrino, and Cushman (1979). A stainless steel stylet was then inserted into the cannula to protect the brain. We ovariectomized females of the same strain using bilateral flank incisions under ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4 mg/kg) anesthesia. They were allowed to recover for at least 1 week before testing. Forty-eight hours prior to the day of testing, females were injected with 10 μg estradiol benzoate and were then injected with 500 μg progesterone 4 hr before testing to induce receptivity and proceptivity. All procedures were in accordance with the National Institutes of Health Guidelines for the Use of Animals and were approved by the local Institutional Animal Care and Use Committee.

Drugs and Procedure

In all experiments, the drug conditions were saline and 100 μg L-NAME (Tocris, Ellisville, MO) dissolved in sterile saline (1.0 μl). This dose was the most effective for Benelli et al. (1995) and Moses and Hull (1999). In Experiment 1A, L-NAME ($n = 6$) and saline ($n = 8$) were microinjected at the rate of 0.5 μl/min through a stainless steel injection cannula that was inserted into the brain 1 mm below the end of the guide cannula. Following the injection, the injection cannula was left in place for 1 min to allow for diffusion into the tissue, and the rats were then placed back into their home cage. A receptive female was introduced into the cage 15 min later, and the rats were allowed to copulate for 30 min after the first intromission. If there was no intromission, the test lasted for 30 min from the introduction of the female. The following behavioral measures were recorded: mount latency (ML), the time from the introduction of the female to the first mount; intromission latency (IL), the time from the introduction of the female to the first intromission; ejaculation latency (EL), the time from the first intromission to the first ejaculation; the total numbers of mounts (MT), intromissions (IT), and ejaculations (ET) in the test; and the postejaculatory interval (PEI), the time from an ejaculation to the first intromission of the next ejaculatory series. In the next 2 weeks, all rats were allowed to copulate with a receptive female every 3 days until they achieved three ejaculations. Only rats that achieved three ejaculations were used in Experiment 1B; thus, 3 rats were excluded for being poor copulators and 2 rats were overdosed with sodium pentobarbitol because they pulled out their cannulas. Therefore, 9 sexually experienced rats were used in Experiment 1B. Experiment 1B administered counterbalanced microinjections of L-NAME and saline 1 week apart and tested the rats as before. In Experiment 2, 42 male rats were separated into three groups: L-NAME ($n = 16$), saline ($n = 9$), and unexposed controls ($n = 17$). Microinjections of saline and L-NAME were administered in the same way as in Experiment 1, and the male was then placed back into his home cage. A sexually receptive female in a wire mesh cage (12.5 cm $\times$ 26.0 cm $\times$ 15.0 cm) was then placed above the male's home cage for 30 min per day for 7 days. During the exposure, the male could smell, see, and hear the female but could not copulate. The 8th day was a drug-free day, on which all male rats were allowed to copulate for 30 min after the first intromission. If there was no intromission, the test lasted for 30 min from the introduction of the female.

After all behavioral testing was completed, rats were euthanized with an overdose of sodium pentobarbital and their brains were removed. Frozen brain sections (40 μm) were cut on a cryostat and slide mounted for verification of correct injector cannula placement. Photomagnifier-enlarged sections showing the ventral extent of cannula damage were compared with atlas drawings from Swanson (2004) to establish proximity to the MPOA (see Figure 1). Only rats with correct cannula placement were included in the experimental groups.

Statistics

In Experiment 1A, an independent sample t test was used to analyze MT, IT, ET, ML, IL, EL, and PEI between the L-NAME and saline groups. Because so few rats copulated following L-NAME injections, latency measures were analyzed only for rats that exhibited the relevant behavior. A chi-square analysis was also performed for the numbers of naive rats mounting, intromitting, and ejaculating. In Experiment 1B, one-way repeated-measures analyses of variance (ANOVAs) were used to compare copulatory behaviors of the L-NAME and saline-treated experienced rats. A two-way chi-square analysis was performed for the numbers of L-NAME and saline-treated experienced rats mounting, intromitting, and ejaculating. In Experiment 2, one-way between-subjects ANOVAs and Bonferroni post hoc tests were used to calculate significant effects of drug on MT, IT, ET, ML, IL, EL, and PEI on all rats on the drug-free test day. Because latency measures were analyzed in all rats for these tests, rats that did not perform a behavior were assigned a latency of 1800 s.

Results

Experiment 1A: Effects of L-NAME on Sexually Naive Male Rats

In Experiment 1A, the 100-μg dose of L-NAME prevented intromissions and ejaculations (see Figure 2). Mounts were recorded in the L-NAME group, although vehicle-treated rats had significantly more mounts, $t(13) = 3.1$, $p < .01$, than rats treated with L-NAME (see Figure 2). L-NAME-treated rats also had longer mount latencies, compared with saline-treated rats, $t(10) = 2.8$, $p < .05$. The results of the chi-square analyses showed that significantly more vehicle-treated rats mounted, $\chi^2(1, N = 14) = 8.9$, $p < .05$, intromitted, $\chi^2(1, N = 14) = 13.7$, $p < .05$, and ejaculated, $\chi^2(1, N = 14) = 12.7$, $p < .05$, than did L-NAME treated rats.

Experiment 1B: Effects of L-NAME on Male Rats With Multiple Copulatory Experiences

In Experiment 1B, significant main effects of drug for numbers of intromissions and ejaculations were found. L-NAME treatment resulted in fewer intromissions, $F(1, 8) = 5.2$, $p < .05$, and ejaculations, $F(1, 8) = 5.8$, $p < .05$, compared with the saline treatment (see Figure 3). There were no significant differences in MT, ML, or IL. The results of the chi-square analysis revealed that the total number of rats that intromitted, $\chi^2(1, N = 9) = 13.6$, $p < .05$, and ejaculated, $\chi^2(1, N = 9) = 13.43$, $p < .05$, was significantly greater in vehicle-treated rats compared with L-NAME, whereas differences in the numbers of rats that mounted were not significant, $\chi^2(1, N = 9) = 4.6$, *ns*. There were too few rats with misplaced cannulas to perform meaningful statistical tests on their data.

Experiment 2: Effects of L-NAME or Saline Microinjections Before Noncopulatory Exposures to a Female

In Experiment 2, one-way between-subjects ANOVAs revealed significant main effects of drug for mount, $F(2, 39) = 6.20$, $p < .01$, intromission, $F(2, 39) = 15.51$, $p < .01$, and ejaculation, $F(2, 39) = 8.09$, $p < .01$, totals (see Figure 4) as well as mount, $F(2, 39) = 5.99$, $p < .01$, intromission, $F(2, 39) = 7.81$, $p < .01$, and ejaculation, $F(2, 39) = 3.57$, $p < .05$, latencies (see Table 1). Bonferroni post hoc analyses revealed that both L-NAME-treated rats and unexposed control rats had fewer intromissions and ejaculations and longer mount and intromission latencies than did the

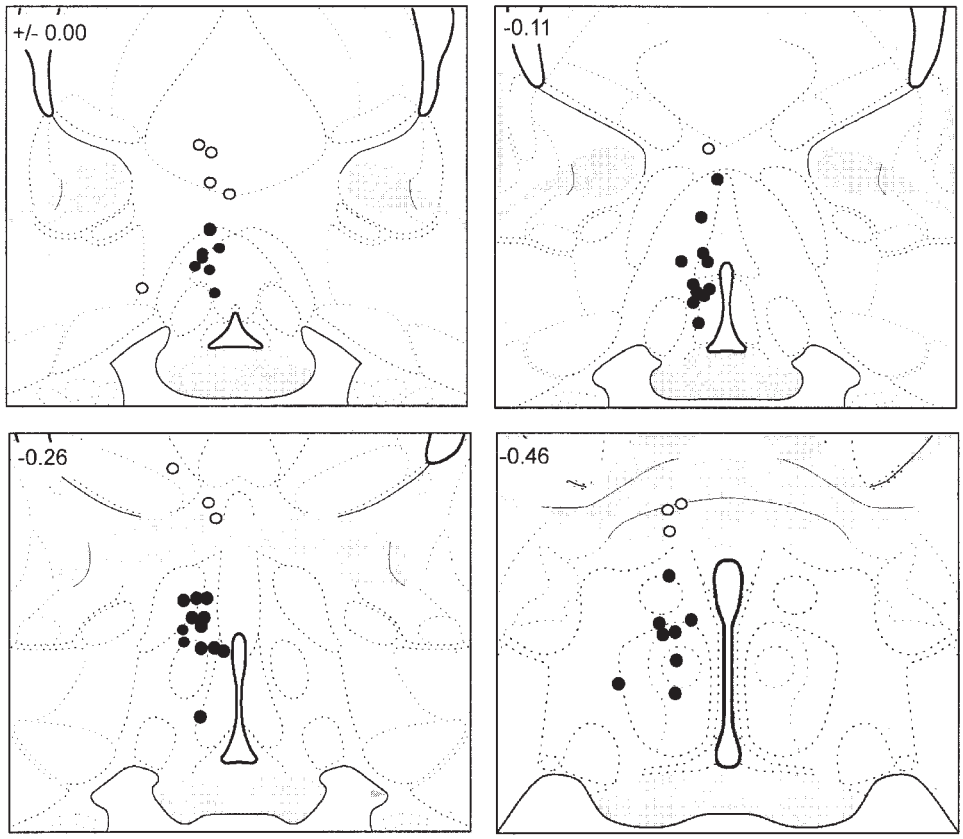

Figure 1. Representative histology depicting cannula placements at the level of the medial preoptic area (MPOA). Filled circles represent placements within the MPOA; empty circles represent those falling outside the MPOA superimposed on figures of Levels 17–20 from Swanson (2004). Reprinted from *Brain maps III: Structure of the rat brain,* 3rd ed., L. W. Swanson, pp. 51, 53, 55, and 57, Copyright (2004), with permission from Elsevier.

saline-treated rats. Post hoc analysis also revealed that L-NAME-treated rats had fewer mounts and longer ejaculation latencies compared with the saline-treated rats. As expected, there were no significant differences between rats treated with L-NAME and unexposed rats. Again, there were too few rats with misplaced cannulas to perform meaningful statistical tests on their data.

Discussion

Inhibition of NO synthesis in the MPOA by L-NAME reduced sexual behavior in male rats. In naive rats, L-NAME significantly decreased the number of mounts and completely prevented intromissions and ejaculations. In experienced rats, L-NAME significantly decreased the numbers of intromissions and ejaculations.

The greater vulnerability of sexually naive males to the inhibitory effects of L-NAME in the MPOA is consistent with previous reports that sexual behavior is more readily disrupted in naive males than in experienced males by castration and various brain lesions (Claro, Segovia, Guillamon, & Del Abril, 1995; de Jonge et al., 1989; Kondo, 1992; Pfaus & Wilkins, 1995; Saito & Moltz, 1986).

In experienced rats in Experiment 1B, neither the number of mounts nor the mount latency was significantly affected by L-NAME. This suggests that L-NAME may not affect sexual motivation or motor activity in experienced males. These results coincide with results of Hull et al. (1994), who found that experienced rats systemically injected with L-NAME chose the female's goal box and ran as fast as saline-treated rats in an X-maze test but had decreased intromissions and ejaculations when allowed to copulate. Thus, even though experienced rats were motivated to copulate, their copulatory behavior was impaired by acute microinjections of L-NAME into the MPOA as well as by systemic L-NAME injections.

Normally, NO is released from both the nerve terminals and the endothelium that lines blood vessels of the penis. It diffuses into smooth cavernosal muscles, causing relaxation and, consequently, erection (Burnett, 1997). Blocking NO synthesis systemically may result in decreased ability to achieve erection, leading to a reduction in copulatory ability. In addition to its role as the major relaxing factor in the periphery (Palmer, Ferrige, & Moncada, 1987), NO may also have central sites of action on erections in at least two brain areas. Sato et al. (1998) reported that administration of L-NMMA into the MPOA by reverse dialysis decreased the rate of mounting by sexually experienced male rats in 10-min copulation tests, and reverse dialysis of the NO precursor, L-arginine,

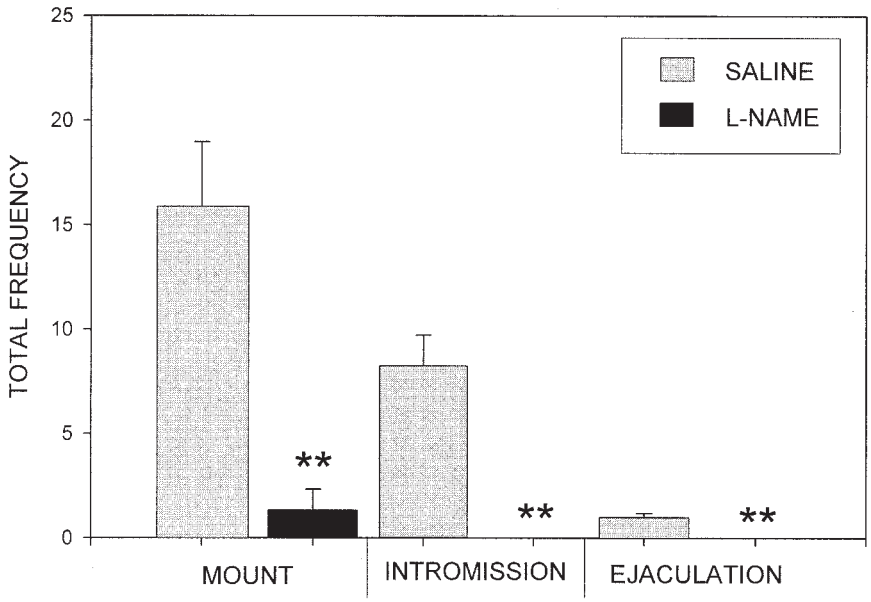

Figure 2. Mean (± *SEM*) total mounts, intromissions, and ejaculations in sexually naive rats treated with L-nitro-arginine methyl ester (L-NAME; 100 μg/μl) or saline. $^{**}p < .01$.

increased mounting rate. In addition, Moses and Hull (1999) found that microinjection of L-NMMA into the MPOA significantly decreased the latency to the first erection or seminal emission and increased the number of seminal emissions in male rats. Both of those effects were hypothesized to result from enhanced sympathetic nervous system activity. The paraventricular nucleus of the hypothalamus (PVN) is another brain area where NO may influence sexual behavior and erection. Reverse dialysis of L-NMMA into the PVN significantly reduced the number of reflexive erections (Sato et al., 1999), and infusion of L-NAME into the PVN reduced noncontact erections and also impaired copulatory behavior in male rats (Melis, Succu, Mauri, & Argiolas, 1998). Our present experiments are consistent with those studies showing that NO promotes sexual function through central as well as peripheral actions. Because the present experiments used a 1-μl volume, and because the cannulas were placed close to the third ventricle, we

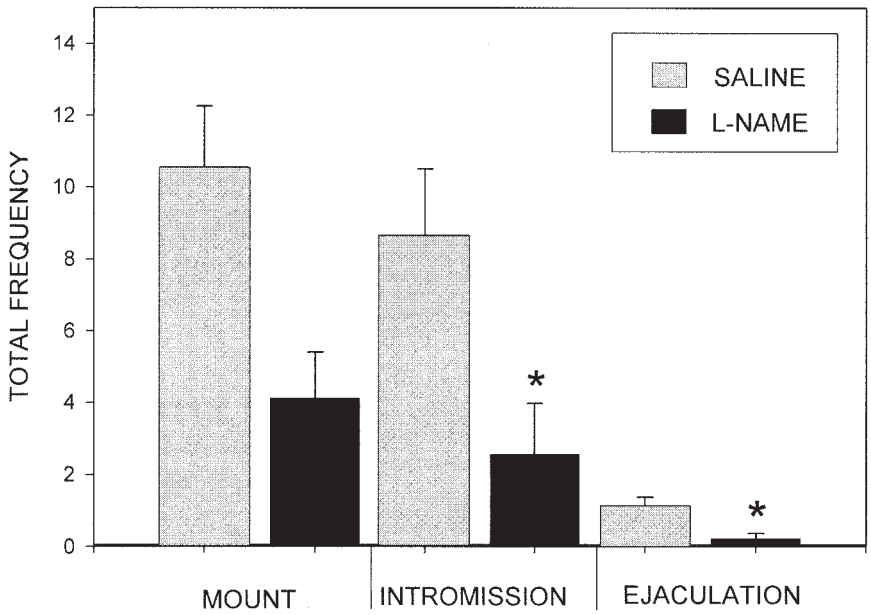

Figure 3. Mean (± *SEM*) total mounts, intromissions, and ejaculations in sexually experienced rats treated with L-nitro-arginine methyl ester (L-NAME; 100 μg/μl) or saline. $^{*}p < .05$.

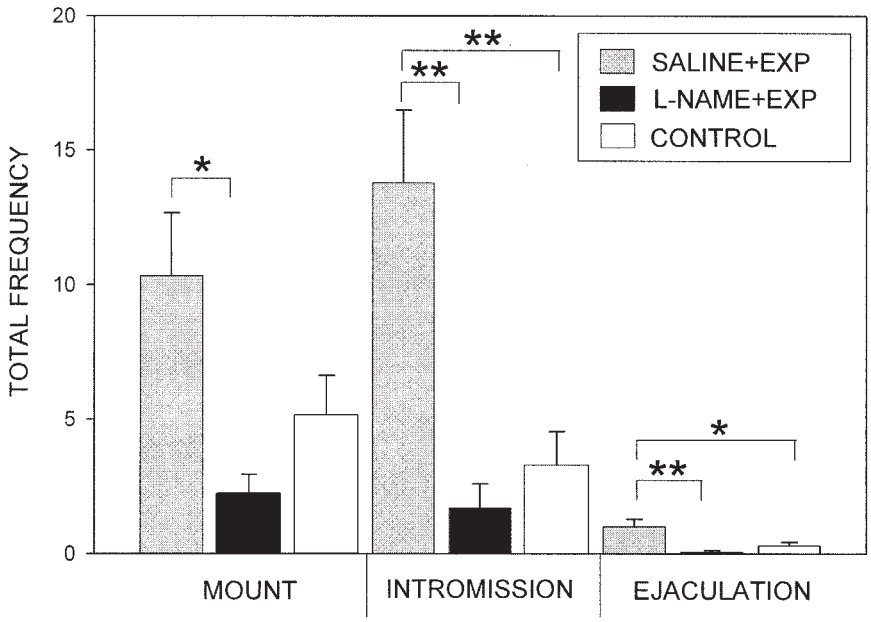

Figure 4. Mean (± *SEM*) total mounts, intromissions, and ejaculations in sexually naive rats treated with L-nitro-arginine methyl ester (L-NAME; 100 $\mu g/\mu l$) or saline before each of seven noncopulatory exposures to a receptive female and tested drug free on Day 8 and in untreated naive males not preexposed to a female. Open bars indicate naive rats that did not receive preexposure, 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. EXP = exposure. *$p < .05$. **$p < .01$.

cannot rule out the possibility that some of our drug leaked into the ventricle and was carried to other brain areas. However, in several rats, dye was microinjected into the cannula prior to removal of the brain for histology; in none of those brains was dye detected in the ventricles. Furthermore, the fact that unilateral reverse microdialysis of smaller amounts of L-NMMA and L-arginine also impaired and facilitated copulation, respectively (Sato et al., 1998), suggests that the MPOA is at least one site for nitrergic control of male sexual behavior.

One way that NO may act centrally is by increasing extracellular levels of neurotransmitters. NO has been reported to increase calcium-dependent and/or calcium-independent vesicular release of dopamine and other neurotransmitters (reviewed in Prast & Philippu, 2001; West, Galloway, & Grace, 2002). NO may also

Table 1
Latency and Frequency Data for Copulatory Measures

Condition	Mount total	Mount latency	Intro. total	Intro. latency	Ejac. total	Ejac. latency	PEI
			Experiment 1A: Naive				
L-NAME ($n = 6$)	1.3 ± 1.0**	1,158.8 ± 301.7	0.0 ± 0.0**	N/A	0.0 ± 0.0**	N/A	N/A
Saline ($n = 8$)	15.9 ± 3.1	158.5 ± 24.1	8.3 ± 1.5	482.4 ± 142.2	1.0 ± 0.2	1,070.4 ± 150.1	657.9 ± 164.6
			Experiment 1B: Experienced				
L-NAME	4.1 ± 1.3	1,017.9 ± 237.1	2.6 ± 1.4*	881.6 ± 233.2	0.22 ± 0.2*	1,649.0 ± 100.1	1,543.7 ± 174.7
Saline	10.6 ± 1.7	502.8 ± 205.9	8.7 ± 1.8	244.4 ± 173.6	1.1 ± 0.3	1,006.0 ± 226.8	798.7 ± 192.1
			Experiment 2: Olfactory exposure				
L-NAME ($n = 16$)	2.3 ± 0.7*[a]	1,037.6 ± 205.7*[a]	1.7 ± 0.9*[a]	1,486.5 ± 169.1*[a]	0.1 ± 0.1*[a]	1,767.1 ± 32.9*[a]	1,725.9 ± 74.1*[a]
Saline ($n = 9$)	10.3 ± 2.3	23.7 ± 7.6*[b]	13.8 ± 2.7*[b]	321.0 ± 199.7*[b]	1.0 ± 0.3*[b]	1,272.9 ± 189.1	968.7 ± 209.3*[b]
Control ($n = 17$)	5.1 ± 1.6	866.5 ± 194.8	3.3 ± 1.3	1,169.6 ± 192.4	0.3 ± 0.1	1,498.7 ± 139.5	1,543.1 ± 125.7

Note. Values are reported as means (± *SEM*). Intro. = intromission; Ejac. = ejaculation; PEI = postejaculatory interval.
* $p < .05$. ** $p < .01$.
[a] Different from saline. [b] Different from control.

inhibit the uptake of dopamine, serotonin, and norepinephrine, thereby prolonging their presence in the extracellular fluid (reviewed in Kiss & Vizi, 2001).

Dopamine in the MPOA is important for sexual behavior in males (reviewed in Hull et al., 2002). Furthermore, dopamine release in the MPOA is regulated by NO. Reverse dialysis of L-arginine, the precursor of NO, into the MPOA increased extracellular dopamine, whereas coadministered L-NMMA blocked the dopamine increase; L-NMMA also decreased basal levels of dopamine when administered alone (Lorrain & Hull, 1993). During a copulation test, L-NAME blocked the normal female-induced increase in extracellular dopamine, whereas the inactive isomer D-NAME did not (Lorrain, Matuszewich, Howard, Du, & Hull, 1996). L-NAME also blocked glutamate-evoked dopamine release in the MPOA (Dominguez, Muschamp, Schmich, & Hull, 2004). However, dopamine's major metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were actually decreased by reverse dialysis of glutamate, and those decreases were also inhibited by L-NAME. The increase in dopamine, together with decreases in its major metabolites, suggests that the dopamine transporter was inhibited by glutamate. Metabolism of dopamine to DOPAC requires transport into the axon terminal, where monoamine oxidase is located on mitochondrial membranes. DOPAC, in turn, may be metabolized to HVA by catechol-O-methyl transferase, which may be located in glia or neurons. Thus, inhibition of the dopamine transporter would decrease the formation of DOPAC and HVA as well as prolong dopamine's presence in the extracellular fluid. Furthermore, the effectiveness of L-NAME in inhibiting both the increase in dopamine and the decreases in its metabolites suggests that NO's inhibition of the transporter contributed to both effects. Thus, NO in the MPOA may function as an intermediary between afferent excitatory input and resident dopaminergic neurons that facilitate copulation.

During copulation, the MPOA integrates relevant sensory information from many brain areas and projects information to structures that program motor output (Simerly & Swanson, 1986, 1988). With this node of the "copulatory circuit" rendered ineffective by NOS inhibition, naive rats are unable to initiate copulation. The requirement of NO for the initial expression of sexual behavior may later be obviated by repeated exposure to estrous females. Over time, female-associated stimuli may sensitize the circuit, permitting more efficient control of the behavior. Thus, although NO appears to be important for the initial acquisition of copulatory behavior, it may not be an absolute requirement for its maintenance. However, even sexually experienced rats in this and other studies have shown sexual deficits with systemically (Benelli et al., 1995; Bialy et al., 1996; Hull et al., 1994) or centrally (Melis et al., 1998; Sato et al., 1998, 1999) administered NOS inhibitors.

Sensitization to sexual stimuli may contribute to sexual behavior in experienced rats. Lumley and Hull (1999) found that copulation to one ejaculation elicited greater Fos-like immunoreactivity (Fos-Li) in the medial preoptic nucleus (MPN) of sexually experienced male rats than in naive rats. This may be due to enhanced effectiveness of stimuli because the increased Fos-Li was observed in spite of a decreased number of intromissions required by experienced rats to trigger ejaculation. Heeb and Yahr (1996) suggested that sexual cues become conditioned after ejaculatory experience, and these sexual cues can increase Fos-Li in the MPN. Neuronal changes, reflected in the increased Fos-Li, may explain the lower susceptibility of experienced rats to exogenous manipulations, such as administration of L-NAME. Thus, the less dramatic effect of L-NAME on experienced rats than on naive rats may be an effect of "sexual conditioning."

Sexual conditioning may occur through chemosensory and other cues from a sexually receptive female. Single (de Jonge et al., 1992) or repeated (Powell et al., 2003) exposures to sexually relevant stimuli facilitate copulatory behavior in males. Our results in Experiment 3 support this finding. Saline-treated rats preexposed to an inaccessible receptive female had increased numbers of intromissions and ejaculations compared with rats that were not preexposed to a female.

NO has been thought to mediate synaptic plasticity and promote long-term potentiation in several brain areas, such as the hippocampus (Doyle, Holscher, Rowan, & Anwyl, 1996; Fin et al., 1995; Schuman & Madison, 1991), the medial vestibular nuclei (Grassi & Pettorossi, 2000), the auditory cortex (Wakatsuki et al., 1998), the forebrain (Barcellos, Bradley, Burns, & Webb, 2000), and the medial amygdala (Abe, Watanabe, & Saito, 1996; Watanabe, Saito, & Abe, 1995). Our findings suggest that the MPOA may be another site where NO can mediate synaptic plasticity and stimulus sensitization. Rats treated with L-NAME before each noncopulatory exposure to a female had fewer mounts, intromissions, and ejaculations, as compared with saline-treated rats, when they were later allowed to copulate drug free. Without sufficient NO synthesis in the MPOA, male rats may not have developed normal sensitization to beneficial female cues that would have later enhanced their copulatory ability.

In summary, acute microinjections of L-NAME into the MPOA decreased the numbers of intromissions and ejaculations in experienced male rats and completely prevented intromissions and ejaculations in naive male rats. Repeated microinjections of L-NAME into the MPOA before each of seven noncopulatory exposures to an estrous female also prevented the improvement that normally would have resulted from such exposures. These results suggest that NO in the MPOA facilitates the initiation of sexual behavior in sexually naive male rats, the progression of copulation in sexually experienced males, and the enhancement of copulation that results from repeated noncopulatory exposures to a female.

References

Abe, K., Watanabe, Y., & Saito, H. (1996). Differential role of nitric oxide in long-term potentiation in the medial and lateral amygdala. *European Journal of Pharmacology, 297,* 43–46.

Barcellos, C. K., Bradley, P. M., Burns, B. D., & Webb, A. C. (2000). Effects of nitric oxide release in the area of the chick forebrain which is essential for early learning. *Developmental Brain Research, 121,* 79–87.

Benelli, A., Bertolini, A., Poggioli, R., Cavazzuit, E., Calza, L., Giardino, L., & Arletti, R. (1995). Nitric oxide is involved in male sexual behavior of rats. *European Journal of Pharmacology, 294,* 505–510.

Bialy, M., Beck, J., Abramczyk, P., Trzebski, A., & Przbylski, J. (1996). Sexual behavior in male rats after nitric oxide synthase inhibition. *Physiology & Behavior, 60,* 139–143.

Burnett, A. L. (1997). Nitric oxide in the penis: Physiology and pathology. *Journal of Urology, 157,* 320–324.

Claro, F., Segovia, S., Guillamon, 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 Research Bulletin, 36,* 1–10.

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 Research Bulletin, 23,* 483–492.

de Jonge, F. H., Oldenburger, W. P., Louwerse, A. L., & van de Poll, N. E. (1992). Changes in male copulatory behavior after sexual exciting stimuli: Effects of medial amygdala lesions. *Physiology & Behavior, 52,* 327–332.

Dominguez, J. M., Muschamp, J. W., Schmich, J. M., & Hull, E. M. (2004). Glutamate-evoked release in the medial preoptic area is mediated by nitric oxide: Implications for male rat sexual behavior. *Neuroscience, 125,* 103–110.

Doyle, C., Holscher, C., Rowan, M. J., & Anwyl, R. (1996). The selective neuronal NO synthase inhibitor 7-nitroindazole blocks both long-term potentiation and depotentiation of field EPSPs in rat hippocampal CA1 in vivo. *Journal of Neuroscience, 16,* 418–424.

Fin, C., Da Cunha, C., Bromberg, E., Schmitz, P. K., Bianchin, M., Medina, J. H., & Izquierdo, I. (1995). Experiments suggesting a role for nitric oxide in the hippocampus in memory process. *Neurobiology of Learning and Memory, 63,* 113–115.

Grassi, S., & Pettorossi, V. E. (2000). Role of nitric oxide in long-term potentiation of the rat medial vestibular nuclei. *Neuroscience, 101,* 157–164.

Hawkins, R. D., Son, H., & Arancio, O. (1998). Nitric oxide as a retrograde messenger during long-term potentiation in hippocampus. *Progress in Brain Research, 118,* 155–172.

Heeb, M. M., & Yahr, P. (1996). *c-fos* immunoreactivity in the sexually dimorphic area of the hypothalamus and related brain regions of male gerbils after exposure to sex-related stimuli or performance of specific sexual behaviors. *Neuroscience, 72,* 1049–1071.

Hull, E. M., Matuszewich, L., Lumley, L. A., Dominguez, J., Moses, J., & Lorrain, D. S. (1994). The roles of nitric oxide in sexual function of male rats. *Neuropharmacology, 33,* 1499–1504.

Hull, E. M., Meisel, R. L., & Sachs, B. D. (2002). Male sexual behavior. In D. W. Pfaff, A. P. Arnold, A. M. Etgen, S. E. Fahrbach, & R. T. Rubin (Eds.), *Hormones, brain and behavior: Vol. 1* (pp. 3–137). New York: Academic Press.

Ignarro, L. J., Bush, P. A., Buga, G. M., Wood, K. S., Fukuto, J. M., & Rajfer, J. (1990). Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle. *Biochemical and Biophysical Research Communications, 170,* 843–850.

Kim, N., Azadzoi, K. M., Goldstein, I., & Saenz de Tejada, I. (1991). A nitric oxide-like factor mediates nonadrenergic–noncholinergic neurogenic relaxation of penile corpus cavernosum smooth muscle. *Journal of Clinical Investigation, 88,* 112–118.

Kiss, J. P., & Vizi, E. S. (2001). Nitric oxide: A novel link between synaptic and nonsynaptic transmission. *Trends in Neuroscience, 24,* 211–215.

Kondo, Y. (1992). Lesions of the medial amygdala produce severe impairment of copulatory behavior in sexually inexperienced male rats. *Physiology & Behavior, 51,* 939–943.

Lorrain, D. S., & Hull, E. M. (1993). Nitric oxide increases dopamine and serotonin release in the medial preoptic area. *NeuroReport, 5,* 87–90.

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.

Lumley, L. A., & Hull, E. M. (1999). Effects of a D1 antagonist and of sexual experience on copulation-induced Fos-like immunoreactivity in the medial preoptic nucleus. *Brain Research, 829,* 55–68.

Melis, M. R., Succu, S., Mauri, A., & Argiolas, A. (1998). Nitric oxide production is increased in the paraventricular nucleus of the hypothalamus of male rats during non-contact penile erections and copulation. *European Journal of Neuroscience, 10,* 1968–1974.

Moses, J., & Hull, E. M. (1999). A nitric oxide synthesis inhibitor administered into the medial preoptic area increases seminal emissions in an ex copula reflex test. *Pharmacology, Biochemistry & Behavior, 63,* 345–348.

Palmer, R. M., Ferrige, A. G., & Moncada, S. (1987, June 11). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature, 327,* 524–526.

Pellegrino, L. J., Pellegrino, A. S., & Cushman, A. J. (1979). *A stereotaxic atlas of the rat brain* (2nd ed.). New York: Academic Press.

Pfaus, J. G., & Wilkins, M. F. (1995). A novel environment disrupts copulation in sexually naive but not experienced male rats: Reversal with naloxone. *Physiology & Behavior, 57,* 1045–1049.

Powell, W., Dominguez, J. M., & Hull, E. M. (2003). An NMDA antagonist blocks the experience-induced enhancement of male sexual behavior. *Behavioral Neuroscience, 117,* 69–75.

Prast, H., & Philippu, A. (2001). Nitric oxide as modulator of neuronal function. *Progress in Neurobiology, 64,* 51–68.

Saito, T. R., & Moltz, H. (1986). Copulatory behavior of sexually naive and sexually experienced male rats following removal of the vomeronasal organ. *Physiology & Behavior, 37,* 507–510.

Sato, Y., Christ, G. J., Horita, H., Adachi, H., Suzuki, N., & Tsukamoto, T. (1999). The effects of alterations in nitric oxide levels in the paraventricular nucleus on copulatory behavior and reflexive erections in male rats. *Journal of Urology, 162,* 2182–2185.

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. *American Journal of Physiology, 274,* R243–R247.

Schuman, E. M., & Madison, D. V. (1991, December 6). A requirement for the intercellular messenger nitric oxide in long-term potentiation. *Science, 254,* 1503–1506.

Simerly, R. B., & Swanson, L. W. (1986). The organization of neural inputs to the medial preoptic nucleus of the rat. *Journal of Comparative Neurology, 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. *Journal of Comparative Neurology, 270,* 209–242.

Swanson, L. W. (2004). *Brain maps III: Structure of the rat brain* (3rd ed.). Elsevier: New York.

Vincent, S., & Kimura, H. (1992). Histochemical mapping of nitric oxide synthase in the rat brain. *Neuroscience, 46,* 111–121.

Wakatsuki, H., Gomi, H., Kudoh, M., Kimura, S., Takahashi, K., Takeda, M., & Shibuki, K. (1998). Layer specific NO dependence of long-term potentiation and biased NO release in layer V in the rat auditory cortex. *Journal of Physiology London, 513,* 71–81.

Watanabe, Y., Saito, H., & Abe, K. (1995). Nitric oxide is involved in long-term potentiation in the medial but not the lateral amygdala neuron synapses in vitro. *Brain Research, 688,* 233–236.

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.

Received January 22, 2004
Revision received May 5, 2004
Accepted June 4, 2004 ■