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An NMDA Antagonist in the MPOA Impairs Copulation and Stimulus Sensitization in Male Rats

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Systemic injections of an NMDA antagonist have been shown to impair mating in male rats. One site where glutamate and its NMDA receptors may contribute to mating is the medial preoptic area (MPOA), which is vital for male sexual behavior. Glutamate is released in the MPOA during copulation, and especially at the time of ejaculation. We report here that the NMDA antagonist MK-801, microinjected into the MPOA, impaired copulatory behavior in sexually naïve as well as experienced males. In rats tested both as naïve and after sexual experience, drug treatment produced more profound impairment in naïve males. In addition, MK-801, microinjected into the MPOA before each of 7 noncopulatory exposures to receptive female rats, resulted in copulatory impairments on a drug-free test on Day 8, relative to aCSF-treated rats; their behavior was similar to that of males that had not been preexposed to females. Therefore, NMDA receptors in the MPOA contribute to the control of copulation and stimulus sensitization. Glutamate, acting via NMDA receptors, regulates many neural functions, including neuronal plasticity. This is the first demonstration that a similar mechanism in the MPOA sensitizes male rats to the stimuli from a receptive female, and thereby enhances their behavior.

Keywords: sexual behavior, medial preoptic area, glutamate, NMDA, MK-801, dizocilpine

Glutamate, acting on N-methyl-D-aspartate (NMDA) receptors, plays a key role in a wide range of physiologic and pathologic processes, including synaptic transmission, neuronal development (Debanne, Daoudal, Sourdet, & Russier, 2003), and multiple aspects of neuronal plasticity and long-term memory (LTM) formation (Barker & Warburton, 2008; Brigman et al., 2010; Delint-Ramirez, Salcedo-Tello, & Bermudez-Rattoni, 2008; Hernandez, Andrzejewski, Sadeghian, Panksepp, & Kelly, 2005; Lee & Everitt, 2008; Popescu, Saghyan, & Paré, 2007; Zhang, Meng, Li, & Han, 2009). Administration of noncompetitive NMDA receptor antagonists such as dizocilpine maleate (MK-801) has been shown to block induction, but not maintenance, of long-term potentiation (LTP) (Gilbert & Mack, 1990).

NMDA receptors also contribute to the facilitative effects of sexual experience on later copulatory proficiency (Fleming & Kucera, 1991; Powell, Dominguez, & Hull, 2003), analogous to their effects on the formation of LTM. However, whereas NMDA antagonists typically affect only the formation, and not the expression, of LTM, systemic administration of an NMDA antagonist did impair the expression of copulation in sexually experienced male rats (Powell et al., 2003). Because glutamate is the major excitatory transmitter throughout the brain, it is not clear where its facilitative effects on male sexual behavior occur.

One possible site where glutamate, acting on NMDA receptors, may normally facilitate male sexual behavior is the medial preoptic area (MPOA). The MPOA is a critical brain area regulating male sexual activity (reviewed in Hull & Rodriguez-Manzo, 2009). The MPOA receives input from (Simerly & Swanson, 1988) and sends projections to (Simerly & Swanson, 1986) other brain areas vital for the initiation and regulation of sexual behavior. Lesions of the MPOA impair mating, and electrical or chemical stimulation of the MPOA enhances copulation and genital reflexes in male rats (reviewed in Hull & Rodriguez-Manzo, 2009). In addition, repeated electrical stimulation of the MPOA

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resulted in copulation by previously inactive males (Paredes, Haller, Manero, Alvaradom, & Ågmo, 1990).

There are several reports of facilitative effects of glutamate in the MPOA on male sexual behavior. In anesthetized male rats, microinjection of glutamate into the MPOA elicited erection (Giuliano et al., 1996) or the urethrogenital reflex (a model of orgasm, Marson & McKenna, 1994). Glutamate is released in the MPOA during copulation and increases dramatically during ejaculation; furthermore, increased glutamate levels, resulting from reverse-dialysis of glutamate uptake inhibitors, facilitated copulation (Dominguez, Gil, & Hull, 2006). Glutamate, reverse-dialyzed into the MPOA, increased dopamine release via a nitric oxide-dependent process (Dominguez, Muschamp, Schmich, & Hull, 2004); MPOA dopamine has also been shown to facilitate male sexual behavior (reviewed in Hull & Rodriguez-Manzo, 2009).

Sexual experience significantly increases the efficiency of copulation. Sexually experienced male rats tend to mount, intromit, and ejaculate faster than inexperienced males (Bialy, Rydz, & Kaczmarek, 2000; Dewsbury, 1969; Larsson, 1978). They also copulate more readily following various lesions or castration, or in a novel environment, compared to naïve rats (de Jonge et al., 1989; Lisk & Heimann, 1980; Pfaus & Wilkins, 1995). Even repeated noncopulatory exposure to an estrous female enhances copulation on the first sexual experience, compared to unexposed naïve controls (de Jonge, Oldenburger, Louwerse, & Van de Pol, 1992; Lagoda, Muschamp, Vigdorchik, & Hull, 2004; Powell et al., 2003). The effects of repeated sexual experience or noncopulatory exposure may result from functional alterations in circuitry mediating male sexual behavior that may in turn enhance responsiveness to sex-related stimuli. However, the neurobiological mechanisms of such stimulus sensitization remain unclear.

The current studies investigated the role of NMDA glutamate receptors in the MPOA in male rat sexual behavior and in stimulus sensitization to sex-related input. In Experiments 1 and 2, we tested the effects of MK-801, microinjected into the MPOA of groups of sexually naive and sexually experienced males, respectively, before copulation tests. In order to test directly the effects of sexual experience as well as of the drug, Experiment 3 tested drug effects in the same males, first when they were naïve and then after they had been given sexual experience. Finally, Experiment 4 tested whether NMDA receptors in the MPOA also play a role in stimulus sensitization resulting from repeated noncopulatory exposures to a female. MK-801 was microinjected into the MPOA of sexually naïve males before each of seven daily noncopulatory exposures to an estrous female rat. Sexual behavior was scored on a drug-free test on the eighth day, thereby avoiding any potential effects of MK-801 on motor behavior.

Method

Subjects

Adult male Long-Evans/Blue Spruce rats weighing 250–300 g (Harlan, Indianapolis, IN), were housed individually in large plastic cages in a temperature- and humidity-controlled environment on a 14:10-hr light–dark cycle, with lights off at 1100 and on at 2100. Food and water were available ad libitum. Rats were weighed daily to ensure health. Ovariectomized female rats of the same strain were used as stimulus rats. All procedures were in accordance with the National Institutes of Health guidelines for the care and use of rats and were approved by the local Institutional Animal Care and Use Committees.

Surgery

Male rats were anesthetized with ketamine hydrochloride (80 mg/kg) and xylazine hydrochloride (10 mg/kg) and then implanted with a 23-g stainless steel guide cannula ending 1 mm above the MPOA. The coordinates with respect to bregma were + 2.1 mm AP, +.4 mm ML, and 7.3 mm DV (Pellegrino, Pellegrino, & Cushman, 1979). Three jeweler's screws were placed in the skull surrounding the cannula, allowing the cannula to be secured when dental acrylic was added. A stylet was placed into the guide cannula after the dental acrylic dried in order to prevent entry of contaminants. After surgery, rats were immediately administered Buprenex (1.5 mg/0.1 ml), and received ground Purina LabDiet rat chow mixed with water for 1 day. Each rat was allowed 1 week for recovery before beginning testing. Females of the same strain were anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4 mg/kg) and ovariectomized using bilateral flank incisions; Buprenex (1.5 mg/0.1 ml) was administered after the surgery. Females were given at least one week after ovariectomy before being used as stimulus rats. They were injected with 10 μg estradiol benzoate 48 hrs prior to testing and with 500 μg of progesterone 4 hrs before testing. Female receptivity was confirmed with a stud male before testing.

Drug

Dizocilpine maleate (MK-801) was obtained from Sigma-Aldrich (St. Louis, MO). For the drug condition, MK-801 was dissolved in aCSF (Dulbecco's solution) at a concentration of 2.5 μg/μl and administered at 0.5 μl/min for a total of 1.0 μl. The dose was the most effective dose used by Maliszewska-Scislo and Trojniar (2000) and did not appear to impair motor behavior in a preliminary mating test. For the vehicle condition, aCSF was administered at 0.5 μl/min for a total of 1.0 μl.

Procedure

Behavior Testing

Copulatory behavior tests were performed in a test room, under dim red light. After several minutes of adaptation, a stimulus female was presented into the male's home cage. The following 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; postejaculatory interval (PEI), the time from an ejaculation to the next intromission; total mount frequency (MF); total intromission frequency (IF); total ejaculation frequency (EF); mount frequency before the first ejaculation (MF_1); intromission frequency before the first ejaculation (IF_1); intromission ratio total (IR_T) and intromission ratio before the first ejaculation (IR_1) [IR = I/(M + I)]; and interintromission interval (III = EL_1/IF_1). If a rat did not ejaculate, MF_1, IF_1, IR_1, and III were not calculated for that animal.

Preliminary Testing of Doses

In a small number of rats, three doses of MK-801 were microinjected into the MPOA, followed by 30 min copulation testing. The treatments were counterbalanced on a weekly basis. The results showed that the highest dose (5.0 μg/μl) produced significant motor and coordination impairment, evidenced by awkward interactions with the female, such as trying to mount from her side. In addition, rats that received this dose became increasingly aggressive. Rats that received the lowest dose (1.0 μg/μl) did not show any abnormal behavior and did not have any motor and coordination impairment. However, this dose did not affect copulation, compared to aCSF treatment. The middle dose (2.5 μg/μl), taken from Maliszewska-Scislo and Trojniar (2000), did not produce any observable motor impairments, but did result in copulatory impairment.

Experiment 1

Twenty sexually naïve male rats were randomly divided into two groups, MK-801 ($n = 10$) and control ($n = 10$). Prior to behavioral testing, the stylet was removed, and the injection cannula was inserted into the guide cannula; it extended 1 mm below the end of the guide cannula into the MPOA. 1.0 μl of drug (2.5 μg/μl) or aCSF was infused unilaterally via a syringe pump at the rate of 0.5 μl/min, with the injection cannula remaining in place for at least 60 sec after the completion of each microinjection to facilitate drug diffusion. Each rat moved freely in his home cage during the injection. To ensure complete diffusion of the drug around the injected area, behavioral testing was not started until 10 min after removal of the injection cannula. Three rats were removed from this study due to incorrect cannula placement, resulting in nine rats remaining in the MK-801 group and eight rats in the aCSF group.

Experiment 2

Methods of Experiment 2 were the same as in Experiment 1, except that prior to testing, rats were allowed to achieve one ejaculation on each of three twice-weekly experience days. If an animal did not ejaculate on one experience day, he was given an additional experience day, so that all males had achieved three ejaculations 1 week before drug versus vehicle testing began. Thus, 30 sexually experienced male rats were divided into two groups, MK-801 ($n = 15$) and aCSF ($n = 15$). MK-801 or aCSF was microinjected into the MPOA in counterbalanced order in two weekly tests, followed by behavioral testing with receptive female rats.

Experiment 3

Twenty-two sexually naïve rats were tested using methods similar to Experiment 1. Two drug-treated males fought repeatedly with the stimulus female, so their tests were ended early. Seven rats had misplaced cannulae; their data were removed, resulting in the following groups: MK-801 ($n = 9$), aCSF ($n = 6$). The rats were then given up to four additional mating experiences over 2 weeks to achieve three ejaculations and become sexually experienced. They were then given two additional mating tests. For the first, they received the opposite treatment from what they had received on their naïve test, and for the second, they received the same treatment as on their naïve test. In other words, if they received drug on the naïve test, they received aCSF on the first postexperience test and drug on the second, and if they received aCSF on the naïve test, they received drug and then aCSF on the two postexperience tests.

Experiment 4. The protocol for this experiment was adapted from Powell et al. (2003) and Lagoda et al., (2004). Sixty sexually naïve male rats were divided into three groups, MK-801 ($n = 20$), aCSF ($n = 20$), and control ($n = 20$). Males in the first two groups received daily microinjections of either MK-801 or aCSF for 7 consecutive days; 10 min after the microinjection, each male was exposed to an inaccessible receptive female rat. The female rat was placed in a wire mesh cage (12.5 × 26 × 15 cm) immediately above the male's cage, thus allowing the male to smell, see and hear her, but not copulate with her. The unexposed control group received daily microinjections of aCSF but were never exposed to females. On the 8th day, rats from all three groups were given drug-free copulation tests with a receptive female for 30 min after the first intromission, or for 30 min if no intromission occurred. Nine rats were removed due to incorrect cannula placement, thus resulting in the following groups: MK-801 ($n = 14$), aCSF ($n = 19$), unexposed control ($n = 18$).

Because of concerns that daily microinjections of MK-801 might compromise the health of the rats, at the end of this experiment, the body weights of drug-treated rats were statistically compared with those of the rats that received aCSF; no significant difference was found between the body weights of the two groups. Since the MPOA also plays an important role in body temperature regulation, we also measured body temperatures of five drug-treated and five aCSF-treated rats, using a rectal thermometer, following the female exposures. No significant difference was found between temperatures of drug and aCSF rats.

Histology

Upon completion of each experiment, rats were anesthetized with a lethal dose of sodium pentobarbital (65 mg/kg) and decapitated. Brains were removed, frozen to −17 °C, and 40 μm coronal sections were cut of the MPOA and any other tissue that contained cannula tracks. Sections were mounted on gelatin-coated slides. Verification of cannula placements was done using a photomagnifier; sections were compared with the images from Brain Maps III (Swanson, 2004). Figure 1 displays cannula placements for all experiments.

Statistics

Experiment 1. Independent-sample *t* tests were used to analyze MF, IF, EF and IR_1. In addition, ML, IL, EL, and III were analyzed only from rats that exhibited the relevant behaviors. Chi-square tests were performed on the numbers of rats that mounted, intromitted, or ejaculated.

Experiment 2. One-way repeated measures analyses of variance (ANOVA) were used to analyze MF, IF, EF, ML, IL, EL and IR_1. As in Experiment 1, latencies for rats that did not show a particular behavior were not analyzed.

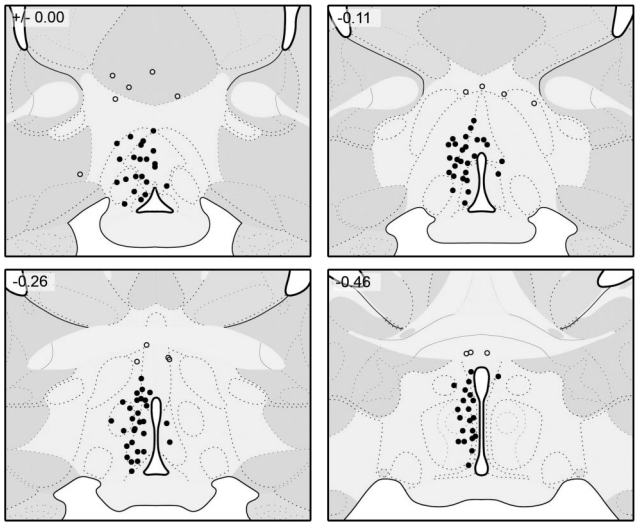

Figure 1. Location of MPOA injection sites in coronal sections based on the atlas by Swanson (2004) for rats in all experiments. *Closed circles* represent accurate MPOA placements. *Open circles* represent cannula placements outside of the MPOA.

Experiment 3. Multiple statistical analyses were used to examine within- and between-subjects effects. Because very few naïve rats copulated after MK-801 injections, latencies to intromit and ejaculate, interintromission interval, and postejaculatory interval could not be analyzed. Chi-square tests were used to compare the numbers of naïve males in the drug and vehicle conditions that mounted, intromitted, or ejaculated.

Two-way ANOVAs compared the effects of drug and experience on frequencies and latencies of mounts, intromissions, and ejaculations. For those comparisons, the data from rats tested with aCSF on the naïve test were compared with data from the aCSF treatment after they had gained experience, and data from rats tested initially with drug on the naïve test were compared with data from the drug test after experience. Individual planned contrast comparisons were also used to probe significant drug effects for separate naïve and experienced tests. Additionally, paired *t* tests were used to compare drug versus aCSF in the counterbalanced tests of experienced males. Chi-square analyses compared the numbers of MK-801- and aCSF-treated experienced males that mounted, intromitted, and ejaculated.

Experiment 4. One-way between-subjects ANOVAs and Bonferroni post hoc tests were used to calculate significance of ML, IL, EL, PEI, MF, IF, EF, and IR_1 on all rats on the drug-free test day. Chi-square tests were performed on the numbers of rats that mounted, intromitted, or ejaculated. For all tests $p < .05$ was considered significant.

Results

Experiment 1

Independent *t* tests revealed significant drug-induced decreases in the numbers of mounts [$t(15) = 3.6$, $p < .01$] and intromissions [$t(15) = 2.14$, $p < .05$] (Figure 2A). Drug-treated males also had lower intromission ratios than did aCSF-treated rats [$t(15) = 3.3$, $p < .01$] (Figure 2A). In addition, rats that received MK-801 had significantly longer mount latencies [$t(15) = 2.3$, $p < .05$] than did aCSF-treated males (Figure 2B). Chi-square analyses revealed that more aCSF-treated rats intromitted [$\chi^2(1) = 3.6$, $p < .05$] and ejaculated [$\chi^2(1) = 7.2$, $p < .01$] than rats that received MK-801.

Experiment 2

Repeated-measures ANOVAs revealed significant decreases in numbers of intromissions [$F(1, 27) = 4.23$, $p < .05$] and ejaculations [$F(1, 27) = 4.31$, $p < .05$ (Figure 3A)] in drug-treated males. Intromission ratios were also significantly decreased [$F(1, 27) = 7.7$, $p < .01$] for the drug-treated rats (Figure 3A). In addition, MK-801 increased mount [$F(1, 20) = 14.44$, $p < .01$], intromission [$F(1, 18) = 19.96$, $p < .01$], and ejaculation [$F(1,$

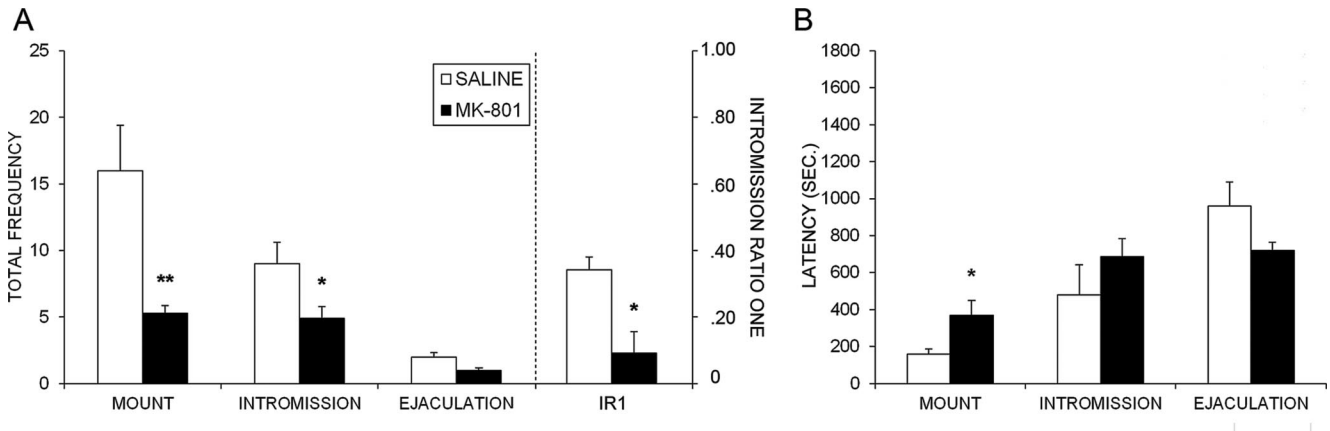

Figure 2. Effects of intra-MPOA administration of MK-801 on copulation in sexually naïve males, Experiment 1. A) Mean (± *SE*) total mounts, intromissions, and ejaculations; and intromission ratio for the first ejaculatory series, in sexually naïve rats treated with MK-801 (2.5 μg/μl) or aCSF in the MPOA. $^{*}p < .05$. $^{**}p < .01$. B) Mean (± *SE*) latencies for mounts, intromissions, and ejaculations in sexually naïve rats treated with MK-801 (2.5 μg/μl) or aCSF in the MPOA. $^{*}p < .05$.

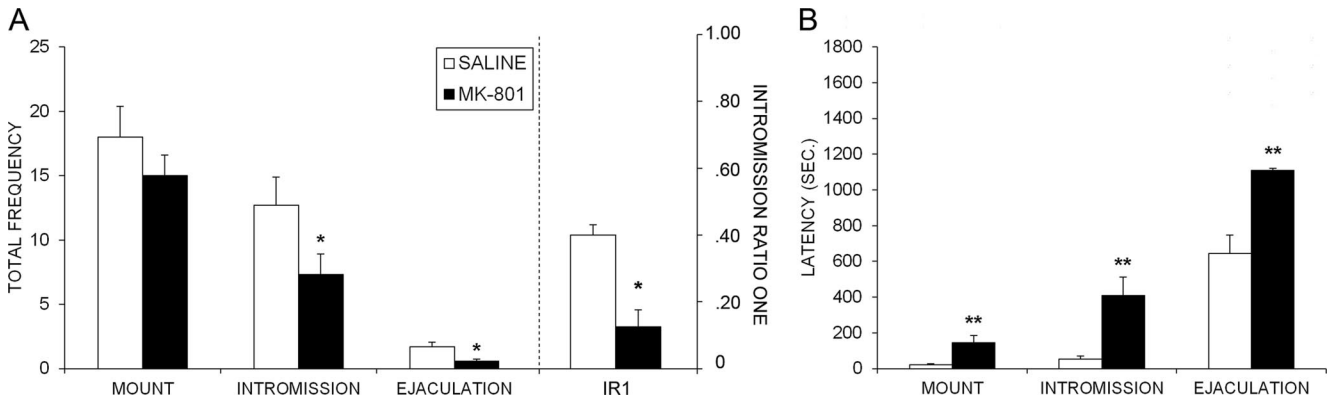

Figure 3. Effects of intra-MPOA administration of MK-801 on copulation in sexually experienced males, Experiment 2. A) Mean (± *SE*) total mounts, intromissions, and ejaculations; and intromission ratio for the first ejaculatory series, in sexually experienced rats treated with MK-801 (2.5 μg/μl) or aCSF in the MPOA. $^{*}p < .05$. B) Mean (± *SE*) latencies for mounts, intromissions, and ejaculations in sexually experienced rats treated with MK-801 (2.5 μg/μl) or aCSF in the MPOA. $^{**}p < .01$.

15) = 8.07, $p < .01$] latencies (Figure 3B). Furthermore, fewer drug-treated rats intromitted [$\chi^2(1) = 13.2, p < .01$] and ejaculated [$\chi^2(1) = 9.3, p < .01$], compared to the aCSF-treated rats.

Experiment 3

Drug-treated naïve males were impaired on several measures. Only three out of nine drug-treated naïve males mounted, compared to six out of six for vehicle-treated rats ($\chi^2 = 4.0, p < .05$); two out of nine intromitted, compared to four out of six for controls [$\chi^2(1) = 5.44, p < .01$]; and one out of nine ejaculated, compared to three of six for controls [$\chi^2(1) = 8.60, p < .01$]. There were no significant differences in mount latency or mount, intromission, or ejaculation frequencies.

Two-way ANOVAs, comparing data from naïve rats with their matched treatment after experience, revealed several significant main effects, but no significant interactions. There was a significant increase in MF as a result of experience [$F(1, 13) = 4.95\ p < .05$], but there was no effect of drug (Figure 4A). For IF, MK-801 decreased intromissions [$F(1, 13) = 7.46, p < .05$], and experience increased intromissions [$F(1/13) = 16.53, p < .001$] (Figure 4B). Similarly, for EF, MK-801 decreased the number of ejaculations [$F(1, 13) = 7.69, p < .02$], whereas experience increased ejaculations [$F(1, 13) = 15.04, p < .01$] (Figure 4C). There were trends for decreased intromission latencies in experienced versus naïve rats [$F(1, 6) = 7.69, p = .05$] and for increased ejaculation latencies in drug-treated versus aCSF-treated rats [$F(1, 3) = 10.45, p = .05$]. Planned contrast comparisons of the means for separate naïve and experienced tests revealed that in naïve males MK-801 significantly decreased the numbers of intromissions [$F(1, 13) = 9.446, p = .009$, Figure 4B] and ejaculations [$F(1, 13) = 5.368, p = .037$, Figure 4C], compared to aCSF treatments. In experienced males there were no significant effects of MK-801, although there was a trend for MK-801 to decrease the number of ejaculations [$F(1, 13) = 3.64, p = .079$], compared to aCSF treatment (Figure 4C). Thus, there were greater effects of MK-801 in rats tested while naïve than after they had gained sexual experience.

Paired *t* tests on postexperience counterbalanced drug and aCSF tests revealed that MK-801 significantly decreased intromission [$t(14) = 3.74, p < .01$] and ejaculation [$t(14) = 3.15, p < .01$] frequencies (Figure 4D), increased mount latencies [$t(10) = 2.25, p < .05$], and produced a trend for increased intromission latencies [$t(10) = 2.03, p = .07$] (Figure 4E). Chi-square tests revealed that sexual experience increased the numbers of drug-treated males that were able to mount [χ^2 (1) = 4.1, $p < .05$], intromit [χ^2 (1) = 5.8, $p < .05$], or ejaculate [χ^2 (1) = 8.9, $p < .01$]; experience did not significantly affect numbers of vehicle-treated males that mounted, intromitted, or ejaculated. Thus, there were complex effects of both drug and experience on sexual behavior.

Experiment 4

MK-801 significantly impaired stimulus sensitization. One-way ANOVAs revealed significant main effects of MK-801 on mount [$F(2, 51) = 5.83, p < .01$], intromission [$F(2, 51) = 9.30, p < .01$], and ejaculation [$F(2, 51) = 5.21, p < .01$] frequencies and on intromission ratio 1 [$F(2/46) = 3.23, p < .05$]. Bonferroni post hoc tests revealed that aCSF-treated exposed rats had significantly more mounts, intromissions, and ejaculations and higher intromission ratios than the MK-801-treated exposed rats (see Figure 5). In addition, control unexposed rats had significantly more mounts than the MK-801-treated exposed rats, but fewer mounts and intromissions than aCSF-treated exposed males.

Chi-square analyses revealed that more aCSF-treated exposed [$\chi^2(1) = 6.17, p < .05$] or control unexposed [$\chi^2(1) = 9.11, p < .01$] rats mounted, compared to MK-801-treated exposed rats. Also significantly more aCSF-treated exposed rats exhibited intromissions than drug-treated exposed [$\chi^2(1) = 10.45, p < .01$] or control unexposed rats [$\chi^2(1) = 4.94, p < .05$]. Finally, more aCSF-treated exposed rats ejaculated, compared to MK-801-treated exposed males [$\chi^2(1) = 8.97, p < .01$].

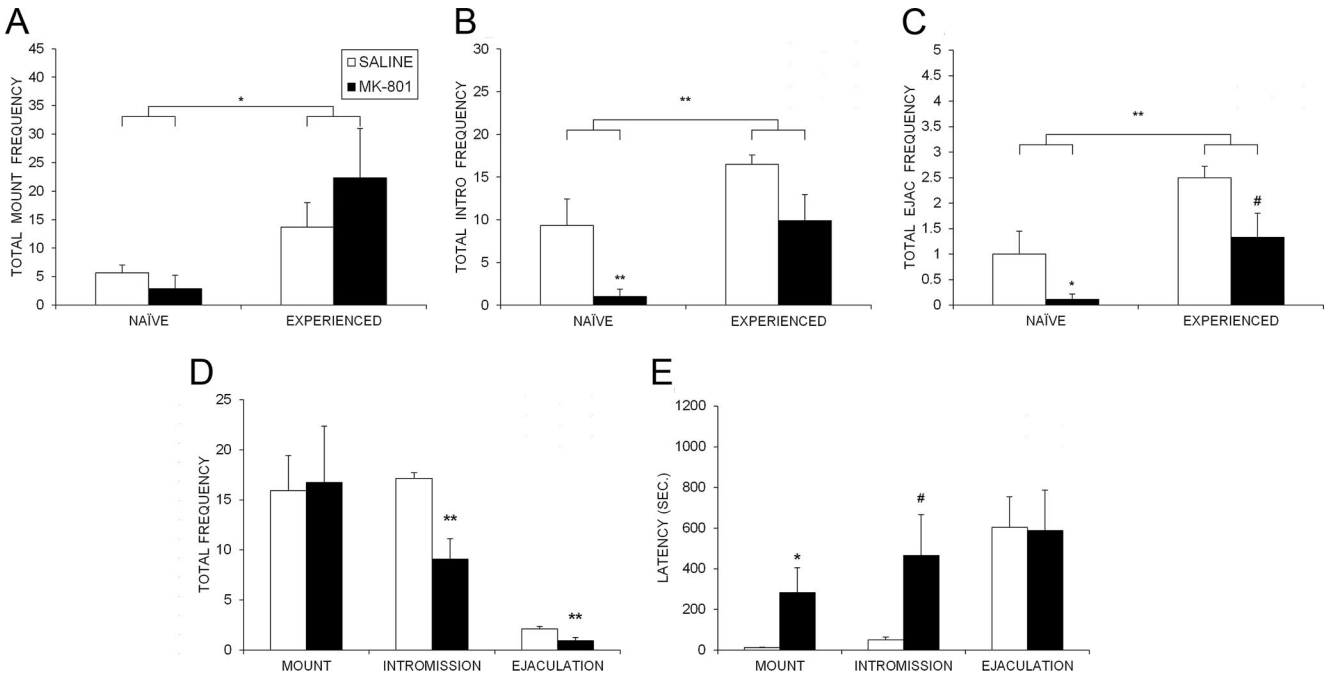

Figure 4. Effects of intra-MPOA administration of MK-801 on copulation before and after repeated sexual experience, Experiment 3. Mean (± *SE*) total: A) mounts, B) intromissions, and C) ejaculations in rats tested when sexually naïve and after three sexual experiences, after treatment with MK-801 (2.5 μg/μl) or aCSF in the MPOA. For rats tested with aCSF when sexually naïve, only data from the aCSF test after experience were analyzed; similarly, for rats tested with drug while naïve, only the drug test after experience was analyzed. Data were analyzed with 2-way ANOVAs with planned comparisons between drug and aCSF in naïve and experienced males. $^{*}p < .05$ for experienced versus naïve rats and drug versus aCSF for naïve rats; $^{**}p < .01$ for experienced versus naïve rats and drug versus aCSF for naïve rats; $\#p = .08$ for drug versus aCSF for experienced rats. D) Mean (± *SE*) total mounts, intromissions, and ejaculations in sexually experienced rats treated with MK-801 (2.5 μg/μl) or aCSF in counterbalanced order. Data were analyzed with a paired *t* test. $^{**}p < .01$. E) Mean (± *SE*) latencies for the first mount, intromission, and ejaculation in sexually experienced rats treated with MK-801 (2.5 μg/μl) or aCSF. Data were analyzed with a paired *t* test. $^{*}p < .05$ for drug versus aCSF for mounts; $\#p = .07$ for drug versus aCSF for intromission latency. Intro = intromission; Ejac = ejaculation.

Discussion

Microinjections of MK-801, a selective and potent noncompetitive antagonist at NMDA receptors (Wong et al., 1986), into the MPOA of male rats decreased the numbers of mounts, intromissions, and/or ejaculations and increased latencies for those measures in sexually naïve and experienced male rats in Experiments 1 and 2. We had expected sexually naïve rats to be more susceptible to the inhibitory effects of MK-801 than sexually experienced rats; however, in Experiments 1 and 2 there was little difference in the effectiveness of the drug in these groups. According to previous reports, sexual behavior in sexually naïve rats is usually disrupted more easily when compared to sexually experienced rats (de Jonge et al., 1989). Also, we have shown in a similar study that sexually naïve rats that received microinjections of the NOS inhibitor L-NAME before a copulation test displayed more deficits in sexual behavior than the same rats after they had received experience (Lagoda et al., 2004). One possible explanation for the apparently similar effectiveness of the drug in the present experiments is the different numbers of rats in Experiments 1 and 2. There were 20 in Experiment 1, half in each group, whereas in Experiment 2 there were 30 that received counterbalanced treatments, resulting in greater statistical power. In our previous experiment with L-NAME, the rats were first tested while sexually naïve; the same rats were then given sexual experience and tested with both drug and aCSF in counterbalanced order. Thus, unlike current Experiments 1 and 2, the naïve and experienced rats in the previous experiment were the same rats.

Therefore, Experiment 3 was designed to test the same rats when they were sexually naïve and again after attaining at least three ejaculations on weekly mating experiences. Indeed, there were greater drug effects when males were naïve than after they had gained experience. Fewer drug-treated naïve rats mounted, intromitted, or ejaculated, compared to controls. However, after the rats had gained sexual experience, there were no significant drug-related differences in numbers of rats performing those behaviors, although there were experience-related differences. Similarly, planned comparison statistical tests revealed significant drug-related decreases in intromissions and ejaculations only in naïve males. More sexually experienced males were able to mount, intromit, and ejaculate, regardless of drug treat-

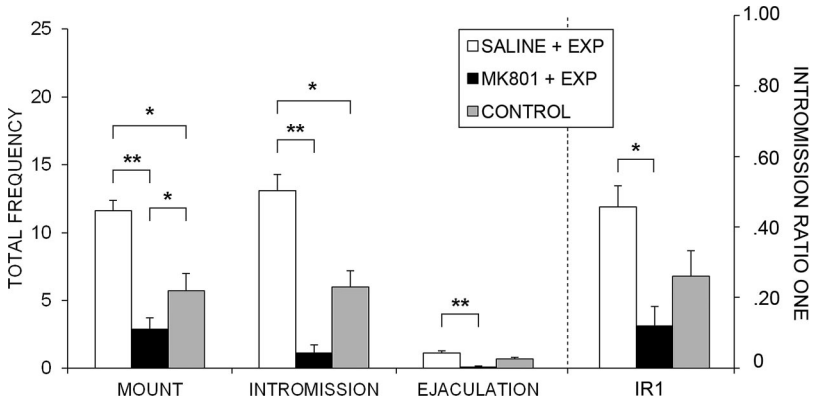

Figure 5. Effects of intra-MPOA administration of MK-801 on stimulus sensitization, Experiment 4. Mean (± *SE*) total mounts, intromissions, and ejaculations; and intromission ratio for the first ejaculatory series, in sexually naive rats treated with MK-801 (2.5 μg/μl) or aCSF before each of seven noncopulatory exposures to a receptive female and tested drug-free on Day 8, and in untreated naïve males not preexposed to a female. Open bars indicate rats that received a microinjection of aCSF before each exposure; black bars indicate rats that received an MK-801 microinjection before each exposure; and gray bars indicate rats that received seven daily microinjections of aCSF, but did not receive any preexposure. $^{*}p < .05$. $^{**}p < .01$.

ment. On the other hand, there were both drug- and experience-related effects on numbers of mounts, intromissions, and ejaculations. MK-801 decreased IF and EF and lengthened EL, while sexual experience increased MF, IF, and EF and decreased IL. Thus, there were complex effects of both drug and experience.

In Experiment 4, microinjections of MK-801 before each of seven daily noncopulatory female exposures significantly impaired rats on the drug-free test on Day 8, compared with aCSF-treated exposed rats. Drug-treated males displayed fewer mounts, intromissions, and ejaculations, compared with the aCSF-treated exposed group and fewer mounts than the unexposed control group. Also, the aCSF-treated exposed group displayed more mounts and intromissions than the unexposed control group. Indeed, these results indicate that NMDA receptors in the MPOA are important for the control of both copulation and stimulus sensitization.

The greater number of mounts by unexposed control rats, compared to MK-801 exposed males, was unexpected. One possible explanation is that there might be some deficit in the basal regulation of normal brain activity of rats repeatedly exposed to MK-801. In other words, basal levels of glutamatergic activity in the MPOA may play an important role in the facilitation of male sexual behavior. By microinjecting MK-801 for 7 consecutive days, glutamate could not stimulate NMDA receptors normally. Thus, repeated antagonism of NMDA receptors may inhibit a mechanism that up-regulates normal responsiveness to sexually relevant stimuli.

There is evidence that repeated systemic administration of MK-801 increased binding of low-affinity nicotinic acetylcholine receptors in the hypothalamus, hippocampus and colliculus of rats and also NMDA binding in the hippocampus (Levin et al., 2005). However, while muscarinic acetylcholine receptors in the MPOA have been shown to reduce the threshold for ejaculation (Hull, Bitran et al., 1988; Hull, Pehek et al., 1988), there is no evidence regarding nicotinic receptors in the MPOA affecting male sexual behavior. Also, 5 and 10 days of systemic MK-801 treatment increased the phosphorylation of enzymes in the frontal cortex of rats that regulate protein synthesis (Yoon et al., 2008) or prosurvival and antiapoptotic activity (Seo et al., 2007). Again, whether local drug administration in the MPOA would have a similar effect is unknown, although it would seem unlikely that such changes would induce the sexual impairment observed in Experiment 4. Finally, in monkey prefrontal cortex there was decreased extracellular glutamate and dopamine after 13 days of intramuscular injections of MK-801 (Tsukada et al., 2005). If a similar effect were produced in the MPOA with local microinjections, that could explain the deficits observed in the present experiments, since both dopamine and glutamate in the MPOA facilitate male sexual behavior (reviewed in Hull & Rodriguez-Manzo, 2009).

Sensitization is an increase in the strength of a specific stimulus following repeated presentations, in this case, the receptive female. Olfaction is critical for successful expression of male reproductive behavior among many rodent species. Olfactory bulbectomy or complete removal of the vomeronasal organ produces deficits in male sexual behavior in rats (Edwards, Griffs, & Tardival, 1990; reviewed in Hull & Rodriguez-Manzo, 2009). Projections from the main and accessory olfactory bulbs travel to the amygdala, which integrates sexually relevant sensory information and relays it to the MPOA via the stria terminalis and the ventral amygdalofugal pathway (Meisel, Lumia, & Sachs, 1980). Furthermore, gonadal steroid receptors in the amygdala, in its projection to the bed nucleus of the stria terminalis (BNST), and in the MPOA enable transmission of the relevant information (reviewed in Newman, 1999; Wood, 1997). A process of consolidation of such sensory input may explain the better sexual performance of rats that received aCSF before exposure to inaccessible receptive females, compared to the rats that received MK-801 before exposure and those that received aCSF but were not exposed to females.

Previous evidence for experience-induced sensitization in the MPOA is provided by Lumley and Hull (1999). Males that had 10

previous copulatory experiences had greater ejaculation-induced immunoreactivity (ir) to c-Fos, the protein product of the immediate-early gene *c-fos*, a measure of neural activity. This occurred despite the fact that the experienced males had somewhat fewer intromissions preceding the ejaculation. Thus, each intromission may have been more effective in the experienced males. Furthermore, activation of NMDA receptors is necessary for mating-induced activation of Fos-ir in the MPOA (Dominguez et al., 2007). The latter study also showed that virtually all Fos-ir neurons in the medial preoptic nucleus (MPN) contained NMDA receptors, and that mating increased phosphorylation of NMDA receptors in the MPN. Finally, it reported that microinjections of MK-801 (1.25 μg/0.25 μl) bilaterally into the MPOA decreased the number of ejaculations and increased ejaculation latency, postejaculatory interval, and mounts preceding ejaculation in sexually experienced males. Therefore, NMDA receptors are important both for performance of mating behavior and for stimulus sensitization.

There are several reports of facilitative effects of glutamate in the MPOA on male sexual behavior. In anesthetized male rats, microinjection of glutamate into the MPOA elicited erection (Giuliano et al., 1996) or the urethrogenital reflex (a model of orgasm, Marson & McKenna, 1994). Glutamate is released in the MPOA during copulation and increases dramatically during ejaculation; and increased glutamate levels, resulting from reverse-dialysis of glutamate uptake inhibitors, facilitated copulation (Dominguez et al., 2006). The glutamate released in the MPOA may arise, at least in part, from glutamatergic projections from the posterodorsal medial amygdala, as has been demonstrated in male gerbils (Simmons & Yahr, 2003).

One way in which NMDA receptors may facilitate sensitization of sexual behavior is by increasing the production of nitric oxide (NO). Neuronal NO synthase (nNOS) is activated by Ca^{2+}, admitted via NMDA receptors (reviewed in Garthwaite, 1991; Luo & Zhu, 2011; Yun, Dawson, & Dawson, 1996). NO has been implicated in the control of male sexual behavior (reviewed in Hull & Rodriguez-Manzo, 2009; Simmons & Yahr, 2011; Succu et al., 2008). We have previously reported that NO is essential for sensitization in an experiment similar to the present one (Lagoda et al., 2004). In addition, glutamate, reverse-dialyzed into the MPOA elicits dopamine release via NO (Dominguez et al., 2004). We have previously reported that dopamine in the MPOA facilitates male sexual behavior (reviewed in Hull & Rodriguez-Manzo, 2009).

One possible explanation for the impairment of sexual behavior of MK-801-treated rats is a decrease in a process similar to LTP, in which rapid stimulation of afferent neurons increases responsiveness of the recipient neurons. This change can last for hours, days, or weeks and may lay a foundation for more permanent changes, such as the construction of new synapses between the neurons (Bennett, 2000). LTP is associated with increased presynaptic neurotransmitter release and enhanced postsynaptic receptor activity, including insertion of AMPA receptors into the postsynaptic membrane (reviewed in Rao & Finkbeiner, 2007; Malenka & Bear, 2004). NMDA receptor channel opening and the resultant calcium influx are necessary for initiating LTP; inhibition of NMDA receptors may impede the cascade of events that would normally lead to consolidation of sexually relevant stimuli in male rats. We suggest that the presence of sexual stimuli, which elicit the release of glutamate in the MPOA (Dominguez et al., 2006), contribute to sensitization to those stimuli and also facilitate copulation.

In summary, acute microinjections of MK-801 into the MPOA decreased the numbers and increased the latencies of mounts, intromissions, and/or ejaculations in sexually naïve and experienced male rats. Repeated microinjections of MK-801 into the MPOA before each of 7 noncopulatory exposures to an estrous female also prevented the improvement that normally would have resulted from such exposures. These results suggest that glutamate in the MPOA facilitates the initiation of sexual behavior in sexually naïve male rats, the progression of copulation in sexually experienced males, and the enhancement of copulation that results from repeated noncopulatory exposures to a female.

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