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# Role of glutamate receptors in the ventromedial hypothalamus in the regulation of female rat sexual behaviors
# I. Behavioral effects of glutamate and its selective receptor agonists AMPA, NMDA and kainate

Michaela Georgescu, James G. Pfaus *

*Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montréal, QC, Canada H4B 1R2*

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**Abstract**

Bilateral infusions of glutamate or kainate to the ventromedial hypothalamus (VMH) have been shown previously to produce a rapid inhibition of lordosis in estrogen-primed female rats induced by manual flank stimulation. The present study examined whether glutamate or selective ionotrophic glutamate receptor agonists can disrupt appetitive and consummatory sexual behaviors following bilateral infusions to the VMH of females during copulation with male rats. Ovariectomized, sexually experienced female rats were implanted bilaterally with guide cannulae aimed at the ventrolateral VMH. After recovery from surgery, females were primed with estradiol benzoate and progesterone and infused with different doses of glutamate, AMPA, NMDA or kainate ($n$=9–10 in each dose group) 3 min prior to a test with sexually vigorous males in bilevel chambers. Glutamate infusions decreased the display of hops and darts and produced a trend toward decreased lordosis. AMPA infusions decreased the display of solicitations, hops and darts, and lordosis. NMDA infusions decreased lordosis and increased defensive behaviors and pacing. Kainate infusions decreased solicitations, hops and darts, and lordosis, and increased defensive behaviors and pacing. These data indicate that the activation of glutamate receptors in the VMH is inhibitory for both appetitive and consummatory aspects of sexual behavior in the female rat.


*Keywords:* Glutamate; Agonists; VMH; Lordosis; Appetitive; Copulation; Estrogen; Progesterone

## 1. Introduction

The sexual behavior of female rats consists of appetitive behaviors such as approach and solicitation, which allow females to be in contact with males; consummatory behaviors such as pacing, which allow the female to set the rate of sexual contact (e.g., control of the interval between intromissions of the penis into the vagina) or that focus the male's copulatory responses, such as hopping and darting, the receptive postural reflex lordosis and defensive behaviors that either enforce a slower rate of sexual contact or terminate sexual behavior altogether (Beach, 1976; Erskine, 1989; Pfaus et al., 1999). Solicitation, hopping and darting, and lordosis lose intensity as copulation progresses through several ejaculatory series, whereas females pace more and engage in more defensive behaviors with successive ejaculations (Pfaus et al., 1999). These female rodent sexual behaviors are largely regulated by the steroid hormones estrogen (E) and progesterone (P) (Beach, 1976).

The ventromedial hypothalamus (VMH) is an important neural site in which E and P act synergistically to regulate sexual behavior (Pfaff, 1980). The ventrolateral region of the VMH is particularly rich in E-concentrating cells (Pfaff and Keiner, 1973; Stumpf, 1970). Lesions of the VMH virtually eliminate the display of lordosis in rodents (Malsbury et al., 1977; Mathews and Edwards, 1977; Pfaff and Sakuma,

* Corresponding author. Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, 7141 Sherbrooke W., Montréal, QC, H4B 1R6 Canada. Fax: +1 514 848 2817.
*E-mail address:* Jim.Pfaus@concordia.ca (J.G. Pfaus).

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1979b), whereas E implants to the VMH of ovariectomized (OVX) females restore their ability to show lordosis (Dörner et al., 1968; Kow and Pfaff, 2004; Rubin and Barfield, 1983b). Implants of P to the VMH of OVX, E-primed rats further facilitate sexual behaviors (Rubin and Barfield, 1983a). Finally, electrical stimulation of this region results in a facilitation of lordosis in E-primed rats (Pfaff and Sakuma, 1979a).

Given that a significant body of evidence points to the presence of lordosis-facilitating neural mechanisms within the VMH, it is surprising that the effect of excitatory agents in this region is inhibitory (Kow et al., 1985; McCarthy et al., 1991). In the first of those studies, bilateral microinfusions of the excitatory amino acid glutamate resulted in a powerful and rapid dose-related inhibition of lordosis induced by manual flank stimulation of ovariectomized females implanted with silastic capsules containing estradiol benzoate (EB) (Kow et al., 1985). The ability to display lordosis recovered 20 min after the infusion. When the glutamate receptor agonist kainate was infused, similar results were obtained except that the effect lasted longer (approximately 4 days for full recovery). Electrophysiological analyses in that study clarified that those drugs excited VMH neurons and that the excitation was fast enough to account for the rapid inhibition of lordosis. In the second of those studies (McCarthy et al., 1991), microinfusions of the glutamate receptor agonist NMDA (*N*-methyl-D-aspartate) resulted in a rapid inhibition of lordosis in rats primed acutely with EB and P and allowed copulatory access to a sexually vigorous male rat. The inhibition was observed 10 min after infusion, but lordosis responses recovered by 30 min. Together, those observations provided evidence of a lordosis-inhibiting neural mechanism by an excitatory agent within the VMH. Since those findings, no other experiments that examine the role of VMH glutamate in female sexual behavior have been published. However, we have recently found that vaginocervical stimulation (VCS) activates Fos protein within glutamate neurons in the VMH (Georgescu et al., submitted for publication). Whether this finding means that glutamate release is involved in the sexual inhibition observed following VCS or simply that glutamate neurons are activated by VCS has not been determined.

The present experiment investigated the role that glutamate receptors in the VMH play in the mediation of female sexual behavior. Most glutamate receptors are ionotropic; that is, the agonist binding sites and associated ion channels are incorporated into the same macromolecular complex. Agonists act to increase the probability that the channel will open. There are three pharmacologically defined classes of ionotropic glutamate receptors, named after their selective agonists: AMPA (á-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid), NMDA and kainate. Although glutamate binding density in the hypothalamus (2.6 pmol/mg protein) is approximately one-third of that in the hippocampus, high densities of glutamate receptors have been detected in the VMH (Meeker et al., 1994); each major glutamate subtype is present in all hypothalamic regions in the following approximate relative densities: NMDA > metabotrophic glutamate receptor > kainate > AMPA.

In the present study, microinfusions of glutamate, or its selective ionotrophic receptor agonists, were administered bilaterally to the VMH of ovariectomized, hormone-primed rats and their effects tested on a full battery of appetitive and consummatory sexual behaviors displayed with sexually vigorous male rats.

## 2. Methods

### 2.1. Subjects

Forty sexually naive female Long–Evans rats, weighing 200 to 250 g, were purchased from Charles River Canada, Inc., St-Constant, QC. Females were housed in groups of four in hanging wire gang cages, but following cannula implantation the females were housed individually in Plexiglas cages with wood-chip bedding. The colony rooms were maintained on a reversed 12:12-h light/dark cycle (lights off at 08:00 h) at approximately 21 °C with food and water continuously available.

Forty Long–Evans males from the same breeder were used as conspecific stimuli. The males received a minimum of 10 prior sexual experiences with receptive females before use and all were sexually vigorous copulators. The males were housed in groups of four in hanging wire-mesh cages in the same colony room.

All animal procedures conformed to the guidelines of the Canadian Council for Animal Care and were approved by the Concordia University Animal Research Ethics Committee.

### 2.2. Surgery

#### 2.2.1. Ovariectomy

Females were OVX bilaterally through lumbar incisions following anesthesia induced by ketamine hydrochloride (50 mg/ml) and xylazine hydrochloride (4 mg/ml), mixed in a ratio of 4:3 respectively and injected intraperitoneally in a volume of 0.8 ml/kg. All OVX rats were given a week of post-surgical recovery before behavioral testing.

#### 2.2.2. Cannula implantation

Females were anesthetized with sodium pentobarbital (65 mg/kg) injected i.p. Females were then implanted bilaterally with double 26-gauge guide cannulae aimed at the ventrolateral VMH using a Kopf stereotaxic instrument. Stereotaxic coordinates were relative to Bregma and implants were made with the head tilted upward at a 5° angle: A–P: 0 mm, M–L: ±1 mm and D–V: −8.5 mm ventral to dura. The tips of the guide cannulae ended 1 mm above the desired target area. Cannula blockers were cut so that they would protrude 0.5 mm from the guide cannula. The 33-gauge infusion cannulae were cut 1 mm longer than the guide cannulae. Guide cannulae, cannulae blockers, injection cannulae and dust caps were obtained from Plastics One (Roanoke, VA). Females were given 7 days of post-surgical recovery before testing.

### 2.3. Hormone and drug administrations

Full sexual receptivity was induced in females by subcutaneous injections of EB (10 μg/0.1 ml reagent grade sesame oil) 48 h and P (500 μg/0.1 ml of sesame oil) 4 h before each test. Steroids were obtained from Steraloids (Hanover, NH).

Drugs were infused in a volume of 1 μl/side using an infusion pump (Harvard Apparatus, Pump 22) set at a rate 1 μl/2 min, as in Kow et al. (1985). Infusion cannulae were left in place for another 1.5 min to promote absorption. Desired concentrations of glutamate (3.3 mmol, 10 mmol, 100 mmol; as in Kow et al., 1985), AMPA (0.3 mmol, 1 mmol, 2 mmol; as in Bell and Kalivas, 1996), NMDA (1 nmol, 3.39 nmol, 6.8 nmol; as in Puma et al., 1996) and kainate (0.469 mmol, 0.938 mmol, 1.17 mmol; as in Kow et al., 1985) were obtained by diluting the drugs in 0.1 M phosphate buffer (pH = 7.0). The physiological saline control solution was also made in 0.1 M phosphate buffer (pH = 7.0) and infused in a volume of 1 μl/side. All drugs were obtained from Sigma (St. Louis, MO).

### 2.4. Behavioral testing

Females received 10 sexual training tests with sexually experienced males at 4-day intervals prior to the study. All training tests were 30 min in duration and were conducted in bilevel chambers (Pfaus et al., 1999) during the middle third of the rats' dark cycle. After the 10th training trial, females were implanted with guide cannulae and allowed 8 days of surgical recovery. However, females were primed with EB and P twice during this period prior to the resumption of behavioral testing. Four hours after the second P priming, females received an infusion of the saline control solution after which they were transferred to the bilevel chambers for a 30-min infusion baseline test of sexual behavior with a sexually vigorous male. This test served as the baseline infusion control for all drug tests. During the next four tests run at 4-day intervals, females were assigned randomly to one of the three dose groups from each drug ($n$ = 9–10/group). Assignment to drug and dose groups was done using a Latin square design so that rats would not receive one type of drug or dose after another in a consistent fashion.

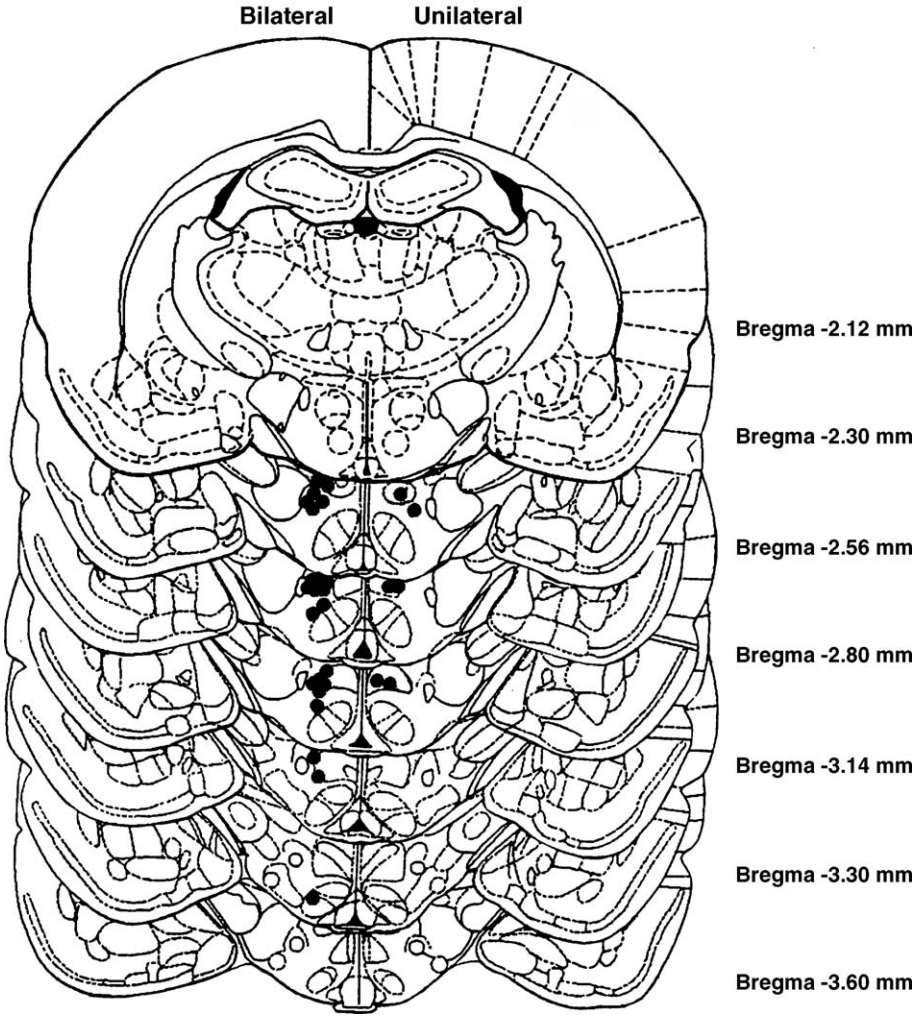


Fig. 1. Guide cannula placements in the present experiments according to the atlas of Paxinos and Watson (1998). Bilateral placements (left side) were retained if guide cannula tracks ended within 1 mm dorsal to the ventrolateral VMH. Unilateral placements (right side) were detected in some animals for which only one cannula track was observed unambiguously. Dots depict the end of the guide cannula. Injection cannula extended 1 mm below the tip of the guides.

All behavioral tests were videotaped and scored subsequently using a computerized event recorder customized for female sexual behavior (Cabilio, 1996). Behavioral data from the first ejaculatory series (from the introduction of the female into the bilevel chamber to the first ejaculation of the male, or the entire 30-min testing period if ejaculation did not occur) were scored, along with the total number of ejaculations received by females during each 30-min test. Behaviors included the frequency of solicitations (characterized by a head-wise orientation of the female towards the male followed by a quick runaway), hops and darts, pacing (frequency of level changes) and rejection responses (boxing, fighting, kicking and prone defensiveness). Lordosis (the dorsoflexion of the back that characterizes sexual receptivity) was analyzed as a lordosis-to-mount quotient (LQ) and a reflex magnitude (LM, on a 1 to 3 scale, as in

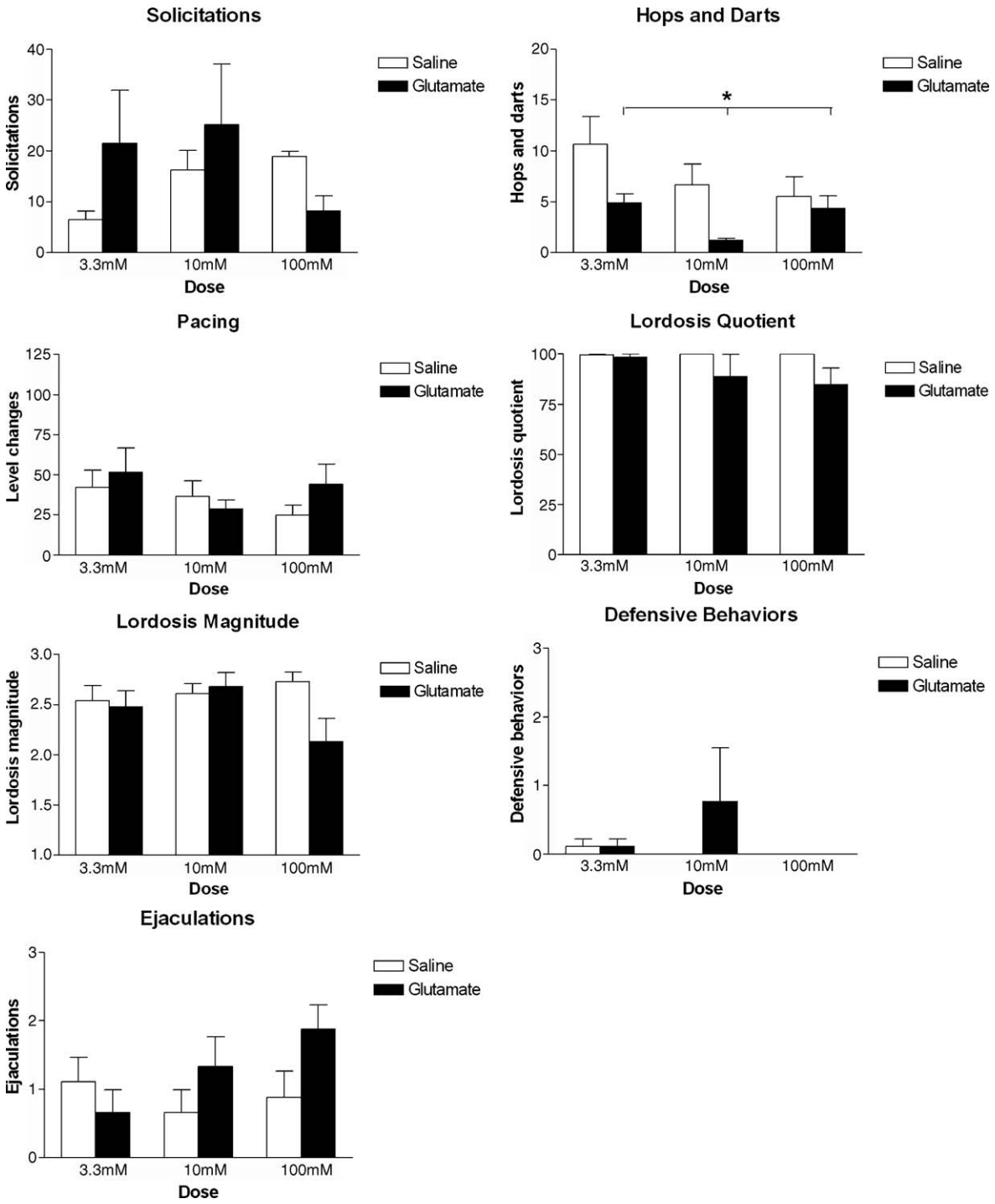


Fig. 2. The effect of glutamate or saline infusions on the number of solicitations, hops and darts, level changes, lordosis quotient and magnitude, defensive behaviors and male ejaculations. Data are means+S.E.M. *$P<0.05$, main effect of drug vs. saline.

Hardy and Debold, 1971), along with the frequency of each magnitude.

### 2.5. Perfusions and histology

Once the final behavioral tests were concluded, females were sacrificed by overdose of sodium pentobarbital (120 mg/kg) in order to verify proper cannulae placement. They were perfused intracardially using a 50-ml syringe filled with ice-cold phosphate-buffered saline followed by 50 ml of 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were removed and placed in a 4% paraformaldehyde solution for 4 h and then into a 30% sucrose solution overnight. Brains were then blocked around the area of the anterior hypothalamus, mounted on a chuck and sliced into coronal sections using a cryostat. Sections were mounted on gel-coated slides, stained with cresyl violet, coverslipped and examined under a microscope to confirm cannulae placements. Animals that died during the study ($n=7$), or that had guide cannula tracks ending outside the anterior or posterior boundaries of the VMH or more than 1 mm dorsal to it ($n=3$), were excluded from the study. Most correct cannula tracks were observed bilaterally ($n=24$), whereas for some we could confirm a cannula track only on one side ($n=6$). We retained these animals for data analysis. Cannula placements are depicted in Fig. 1.

### 2.6. Statistical analyses

Separate 3 (dose: small, medium, large) × 2 (treatment: drug vs. saline) analyses of variance (ANOVAs) were conducted with dose as a between-measures variable and treatment as a repeated-measures variable for all sexual behaviors in each drug condition. In each case, the saline trial served as the baseline measure to which drug measures were compared. For each significant interaction, Tukey post hoc comparisons of the individual means were conducted, $P<0.05$.

## 3. Results

### 3.1. General observations

Females did not require any special handling following infusions of glutamate or AMPA and no hyperactivity was observed. However, following infusions of kainate or NMDA, a small number of females were difficult to handle and evidence of hyperactivity was observed (e.g., those females spent large portions of the test sessions engaged in level changing or chewing on the walls or metal floor grids of the bilevel chamber).

### 3.2. Effects of glutamate

Fig. 2 shows the effects of infusions of saline and the three doses of glutamate in the VMH on female sexual behaviors and male ejaculatory responses. The ANOVA detected a significant main effect of treatment only on the number of hops and darts, $F(1,24)=10.43$, $P<0.01$. Post hoc comparisons revealed that animals displayed fewer hops and darts when infused with glutamate compared to saline. Glutamate infusions had no significant effects on the other measures.

### 3.3. Effects of kainate

Fig. 3 shows the effects of infusions of saline and 3 doses of kainate on female sexual behaviors and male ejaculatory responses. Overall, kainate decreased the number of solicitations, hops and darts, LQs and LMs, increased pacing and defensive behaviors, but had no effect on male ejaculations.

#### 3.3.1. Solicitations

The ANOVA detected a significant main effect of treatment, $F(1,23)=4.90$, $P<0.05$. Post hoc analyses revealed that kainate treatment overall reduced solicitations compared with saline treatment.

#### 3.3.2. Hops and darts

The ANOVA detected a significant interaction between dose and treatment, $F(2,23)=4.23$, $P<0.05$. Post hoc analyses revealed that females infused with the largest dose displayed significantly fewer hops and darts compared to saline infusions.

#### 3.3.3. Pacing

A significant main effect of treatment was detected, $F(1,23)=10.52$, $P<0.01$. Post hoc analyses revealed that animals infused kainate engaged in more level changing overall compared with saline treatment.

#### 3.3.4. Lordosis quotient

The ANOVA detected a significant main effect of treatment, $F(1,23)=16.22$, $P<0.001$. Post hoc analyses revealed that kainate treatment overall decreased LQs relative to saline treatment.

No significant main effects of dose or treatment, or their interaction, were detected for other measures.

### 3.4. Effects of AMPA

Fig. 4 shows the effects of infusions of saline and three doses of AMPA on female sexual behaviors and male ejaculations. The effects of AMPA were similar to those of kainate. Overall, infusions of AMPA decreased the number of solicitations, hops and darts, LQs and LMs, and increased the number of defensive behaviors, but had no significant effect on the number of level changes or ejaculations.

#### 3.4.1. Solicitations

The ANOVA detected a significant main effect of treatment, $F(1,25)=14.99$, $P<0.001$. Post hoc analyses revealing that animals treated with AMPA displayed significantly fewer solicitations overall compared to saline treatment.

*3.4.2. Hops and darts*

A significant main effect of treatment was found, $F(1,25) = 4.32$, $P < 0.05$. Post hoc analyses revealed that animals treated with AMPA displayed significantly fewer hops and darts overall compared to saline treatment.

*3.4.3. Lordosis quotient*

A significant main effect of treatment was detected, $F(1,25) = 13.91$, $P < 0.001$. Post hoc analyses revealed that AMPA treatment overall decreased LQs significantly compared to saline.

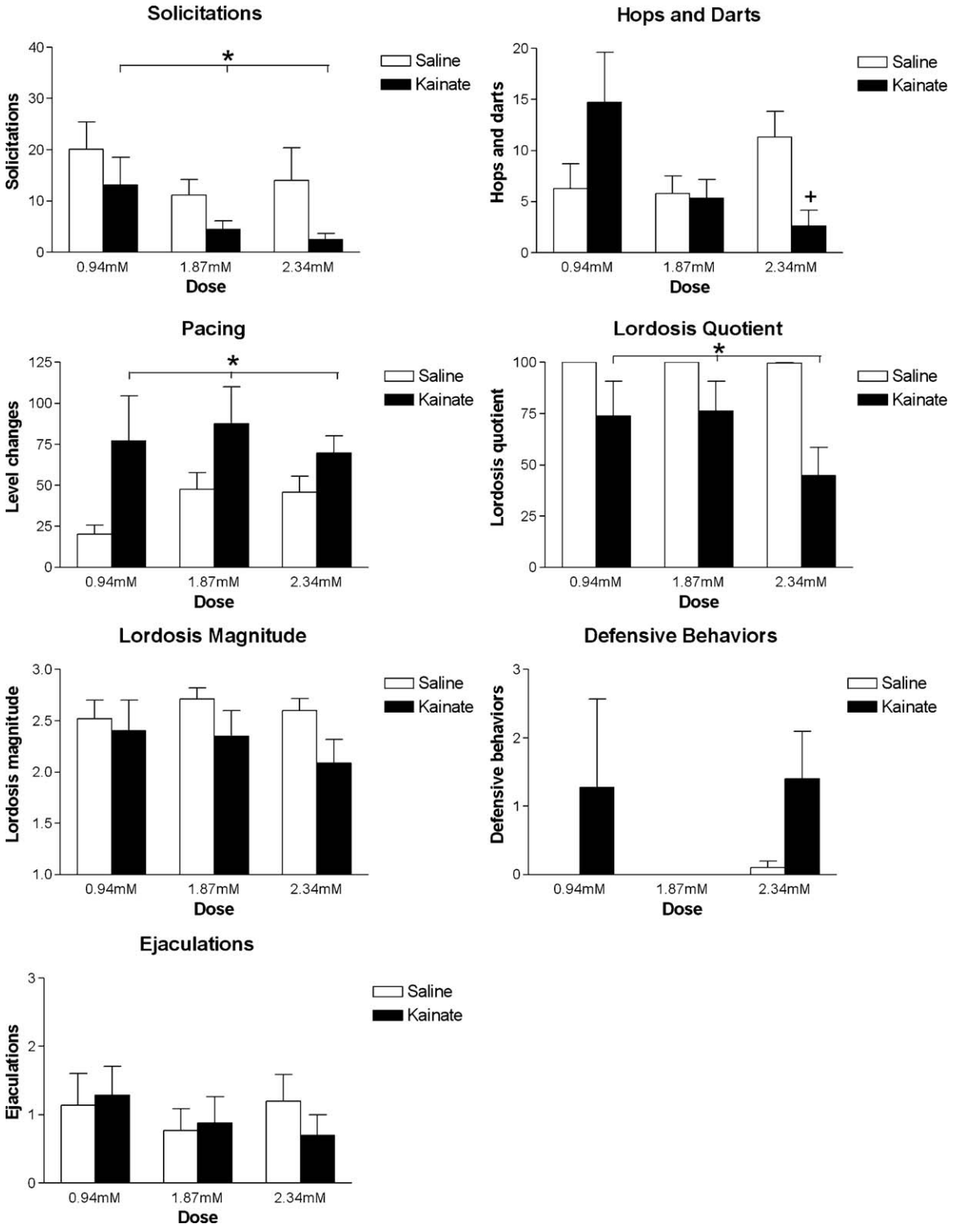


Fig. 3. The effect of kainate or saline infusions on the number of solicitations, hops and darts, level changes, lordosis quotient and magnitude, defensive behaviors and male ejaculations. Data are means + S.E.M. *$P < 0.05$, main effect of drug vs. saline. $^{+}P < 0.05$, post hoc Tukey test of the high dose of kainate vs. saline.

*3.4.4. Lordosis magnitude*

The ANOVA detected a significant main effect of treatment, $F(1,19)=15.97$, $P<0.001$. Post hoc analyses revealed that animals treated with AMPA had lower LMs overall compared to saline treatment.

*3.4.5. Defensive behaviors*

The ANOVA detected a significant main effect of dose, $F(2,25)=3.59$, $P<0.05$. Post hoc analyses revealed that animals that received the low dose engaged in significantly more defensive behaviors overall than animals that received the medium or high doses.

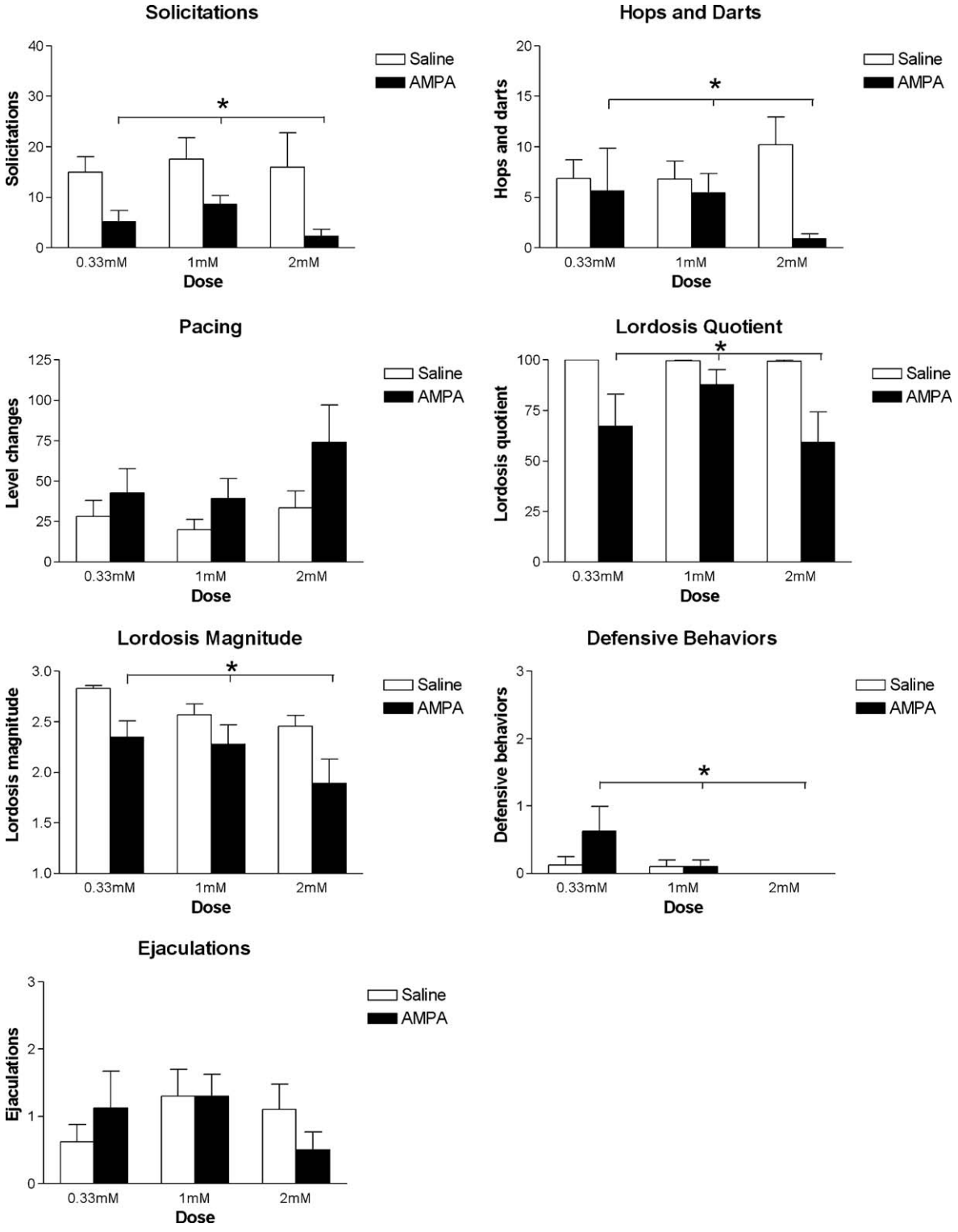


Fig. 4. The effect of AMPA or saline infusions on the number of solicitations, hops and darts, level changes, lordosis quotient and magnitude, defensive behaviors and male ejaculations. Data are means+S.E.M. *$P<0.05$, main effect of drug vs. saline.

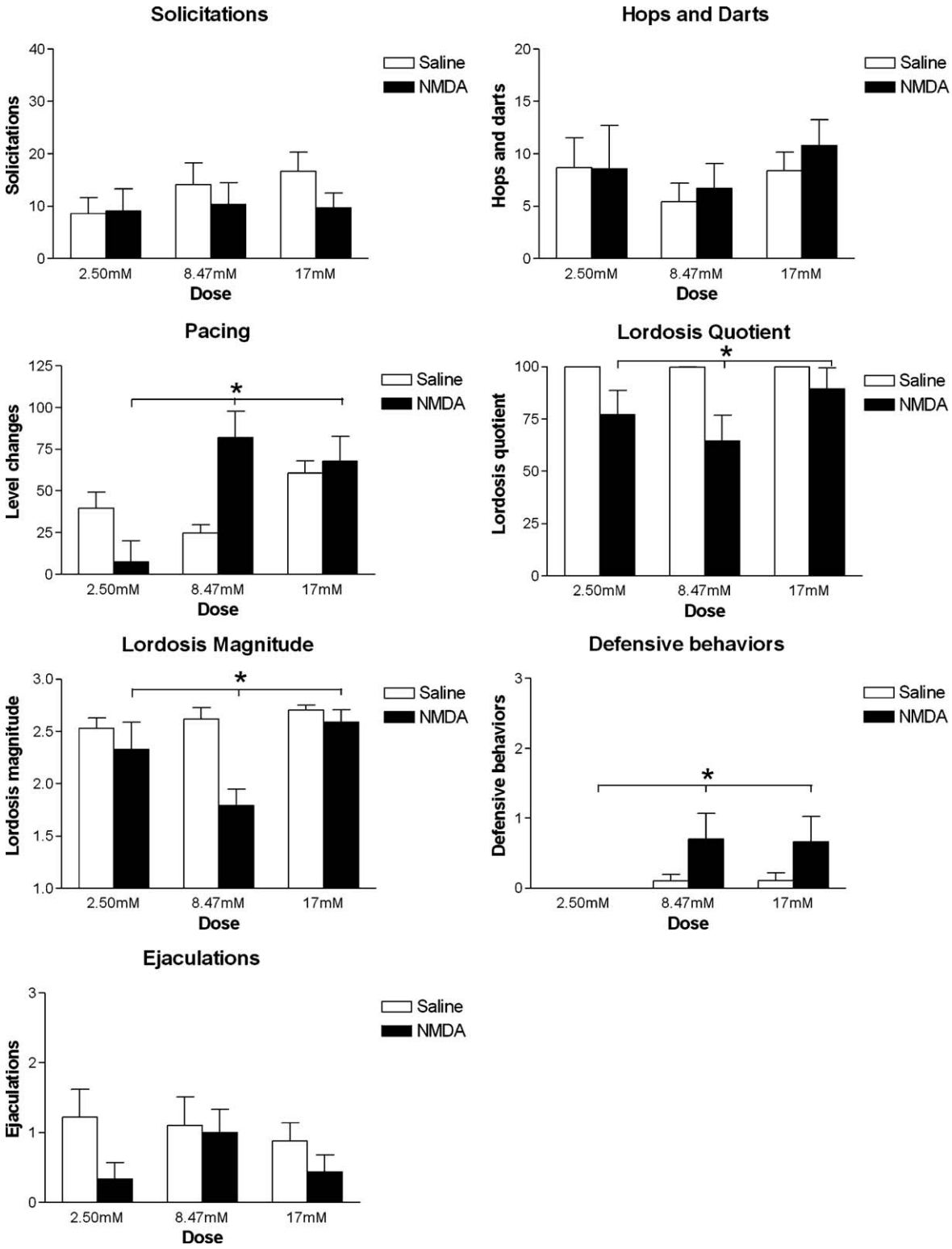


Fig. 5. The effect of NMDA or saline infusions on the number of solicitations, hops and darts, level changes, lordosis quotient and magnitude, defensive behaviors and male ejaculations. Data are means+S.E.M. *$P<0.05$, main effect of drug vs. saline.

No significant main effects of dose or treatment, or their interaction, were detected for other measures.

### 3.5. Effects of NMDA

Fig. 5 shows the effects of infusions of saline and the three doses of NMDA on female sexual behaviors and male ejaculatory responses to the females. Overall, NMDA infusions increased pacing and the number of defensive behaviors, and decreased LQs and LMs, but had no effect on the number of solicitations or hops and darts.

#### 3.5.1. Pacing

The ANOVA detected a significant main effect of treatment, $F(1,25)=16.32$, $P<0.001$. Post hoc analyses revealed that animals treated with NMDA displayed significantly more level changes overall compared to saline treatment.

#### 3.5.2. Lordosis quotient

A significant main effect of treatment was detected, $F(1,25)=11.84$, $P<0.01$. Post hoc analyses revealed that animals treated with AMPA had significantly lower LQs compared to saline treatment.

#### 3.5.3. Lordosis magnitude

The ANOVA detected a significant main effect of treatment, $F(1,23)=7.89$, $P<.01$. Post hoc analyses revealed that rats infused with NMDA overall had significantly lower LMs compared to saline treatment. The ANOVA also detected a significant main effect of dose, $F(2,23)=5.71$, $P<0.01$. Post hoc analyses revealed that infusions of the medium dose resulted in significantly lower LMs compared to the low and high doses.

#### 3.5.4. Defensive behaviors

The ANOVA detected a significant main effect of treatment, $F(1,25)=4.21$, $P<0.01$. Post hoc analyses revealed that animals treated with NMDA displayed significantly more defensive behaviors overall compared to saline treatment.

No significant main effects of dose or treatment, or their interaction, were detected for other measures.

## 4. Discussion

Previous research reported that the excitatory neurotransmitter glutamate, or one of its selective ionotrophic receptor agonists kainate, inhibits lordosis when administered bilaterally to the VMH of female rats that receive manual stimulation of the flanks (Kow et al., 1985), or copulatory stimulation by a male rat (McCarthy et al., 1991). The purpose of the present study was to further investigate the role of glutamate receptors within the VMH by analyzing the effects of infusions of glutamate or three selective receptor agonists, AMPA, NMDA and kainate, on appetitive and consummatory aspects of female sexual behavior when females were paired with sexually active male rats. The results confirm and extend those of Kow et al. and McCarthy et al., and indicate that glutamate receptors within the VMH play an inhibitory role, not only in the regulation of lordosis, but also in the regulation of other appetitive and consummatory sexual behaviors.

Although glutamate infusions reduced the number of hops and darts, they did not have an inhibitory effect on lordosis. At first glance, this would appear contrary to the original findings of Kow et al. (1985). However, there are at least three differences in methodology between the present experiments and those of Kow et al. that could explain this discrepancy. First, Kow et al. primed OVX rats with EB-filled silastic capsules implanted subcutaneously. This hormone replacement regimen induces a lower level of sexual receptivity than full priming with EB and P. Perhaps, the low levels of lordosis induced by EB alone are more easily disrupted by glutamate than high levels of lordosis, or perhaps the addition of P in the present experiment buffered females from the effects of glutamate. Second, in the present experiment, lordosis was induced by stimulation from a sexually vigorous male compared to the manual flank stimulation used by Kow et al. Perhaps, female rats are less responsive to glutamate infusions to the VMH when they receive multi-sensory stimulation from a sexually vigorous male (including olfactory, flank and vagino-cervical stimulation), relative to manual flank stimulation alone. Third, the females in the present study had extensive sexual experience before testing. Sexual experience is known to buffer male rats from treatments that disrupt sexual behavior (Pfaus and Wilkins, 1995) and may do so in females. It is not clear how much prior sexual experience females had in the Kow et al. study.

Infusions of kainate to the VMH had more pronounced effects on sexual behavior compared to glutamate. For example, the number of solicitations decreased significantly, the number of hops and darts was decreased in a dose-related fashion, lordosis quotients were decreased, and there were trends toward a decrease in lordosis magnitude and an increase in defensive behaviors. Moreover, pacing was increased significantly and substantially following kainate infusions, suggesting that females were trying to avoid sexual contact. Although kainate is highly excitotoxic to the brain, the largest dose administered was approximately 10 times smaller than that necessary to induce lesions (Stubley-Weatherly et al., 1996). One study indicated that the effect of infusions of a subtoxic dose (60 pmol) of kainate to the VMH induces a defense reaction characterized by increased locomotion, rearing and leaping (Silveira and Graeff, 1992); increased locomotion and leaping were also observed following infusions of kainate in the present experiment. It is therefore possible that the general neuronal excitability induced by kainate and its effects on locomotion in general caused the reduction in sexual behavior observed in the present study.

Similar to the results of McCarthy et al. (1991), infusions of NMDA resulted in dose-related decreases in lordosis magnitude, decreases in lordosis quotients, and increases in defensive behaviors and pacing. Frequencies of solicitation, hopping and darting and ejaculation by the males were not significantly affected by NMDA, indicating that attractiveness of the female to the male was preserved. These results suggest that NMDA

receptors within the VMH may be involved in the inhibition of lordosis and overall pacing of the rate of copulation, characterized by level changing and defensive responses by the females.

Infusions of AMPA produced significant decreases in solicitation, hopping and darting, lordosis magnitude and lordosis quotient; however, these infusions had no effect on the number of ejaculations by the male or on female defensive behaviors and pacing. This pattern of data suggests that AMPA receptors within the VMH may mediate both appetitive and consummatory sexual behaviors, but not pacing or the display of defensive behaviors.

Although glutamate binds to all three ionotrophic receptor subtypes and its G-protein coupled metabotrophic receptor, the behavioral effect of glutamate reported in this study was less than the sum of its parts. Clearly, AMPA, NMDA and kainate had more pronounced effects. This is similar to the weaker action of glutamate relative to its ionotrophic agonists in producing excitotoxic and electrophysiological effects (Grimwood et al., 1991; Radecka et al., 1999; Vornov et al., 1995). Part of this may stem from the fact that glutamate uptake into glial cells from the extracellular space is highly efficient (e.g., Bergles and Jahr, 1998) and can prevent the excitotoxic effects of high concentrations (Tanaka et al., 1997). Thus, the glutamate infused here may have been depleted relatively rapidly from the extracellular space, whereas the agonists likely took longer to be degraded, resulting in a more pronounced effect. Based on this, we suggest that glutamate release in the VMH must occur in a tonic manner to produce behavioral effects.

Taken together, the pattern of data observed in this study shows that activation of glutamate receptors in the VMH inhibits both appetitive and consummatory sexual behaviors in the female rat, and stimulates pacing and defensive responses. This pattern of effect is reminiscent of the behavioral pattern observed during estrus termination. VCS induced by multiple penile intromissions and ejaculations, or manually by a lubricated glass rod, leads to a faster termination of estrus (Lodder and Zeilmaker, 1976) that is characterized by an early inhibition of appetitive behaviors, such as solicitation and hops and darts, a facilitation of pacing and rejection responses, followed in time by an inhibition of lordosis (Pfaus et al., 2000). We reported previously that increasing amounts of VCS increase Fos progressively in a cluster of neurons in the ventrolateral VMH (Pfaus et al., 1996). This activation was lower in OVX rats treated with estrogen and progesterone compared to rats treated with the oil vehicle when females received 1, 5, 10 or 20 VCSs, but not 30, 40 or 50 (which reflects the total approximate number of intromissions they would receive in an hour of copulation with a male rat). Those data suggest that estrogen blunts the ability of lower amounts of VCS to activate this cluster of neurons in the VMH, but that larger amounts of VCS override this effect. We have recently found that a large percentage of the Fos-positive neurons activated by VCS in the VMH are co-labelled for glutamate (Georgescu et al., submitted for publication). Given the present findings, we speculate that the activation of glutamate neurons in the VMH by VCS may be a critical neurochemical mediator of estrous termination. Thus, in addition to its facilitative actions on lordosis (Pfaff, 1980; Pfaff and Sakuma, 1979a,b), estrogens may alter the function of the VMH to promote both appetitive and consummatory female sexual behaviors by blunting the ability of VCS to activate this glutamate system.

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## References

Beach FA. Sexual attractivity, proceptivity and receptivity in female mammals. Horm Behav 1976;7:105–38.

Bell K, Kalivas PW. Context-specific cross-sensitization between systemic cocaine and intra-accumbens AMPA infusion in the rat. Psychopharmacology 1996;127:377–83.

Bergles ED, Jahr CE. Glial contribution to glutamate uptake at Schaffer collateral-commissural synapses in the hippocampus. J Neurosci 1998;18:7709–16.

Cabilio S. 1996. Rodent Sex Behavior Scoring Program. Unpublished computer software program, Concordia University.

Dörner G, Docke F, Moustafa S. Differential localization of a male and a female hypothalamic mating center. J Reprod Fertil 1968;17:583–6.

Erskine MS. Solicitation behavior in the estrous female rat: a review. Horm Behav 1989;23:473–502.

Georgescu M, Del Corpo A, Sabongui C, Pfaus JG. submitted for publication. Vaginocervical stimulation activates glutamate neurons in the ventromedial hypothalamus: attenuation by estrogen.

Grimwood S, Foster AC, Kemp JA. The pharmacological specificity of *N*-methyl-D-aspartate receptors in rat cerebral cortex: correspondence between radioligand binding and electrophysiological measurements. Br J Pharmacol 1991;103:1382–95.

Hardy DF, Debold JF. Effects of mounts without intromission upon the behavior of female rats during the onset of estrogen-induced heat. Physiol Behav 1971;7:643–5.

Kow L-M, Pfaff DW. The membrane actions of estrogens can potentiate their lordosis behavior-facilitating genomic actions. Proc Natl Acad Sci U S A 2004;101:12354–7.

Kow L-M, Harlan RE, Shivers BD, Pfaff DW. Inhibition of the lordosis reflex in rats by intrahypothalamic infusion of neural excitatory agents: evidence that the hypothalamus contains separate inhibitory and facilitatory elements. Brain Res 1985;341:26–34.

Lodder J, Zeilmaker GH. Role of pelvic nerves in the postcopulatory abbreviation of behavioral estrus in female rats. J Comp Physiol Psychol 1976;90:925–9.

Malsbury CW, Kow L-M, Pfaff DW. Effects of medial hypothalamic lesions on the lordosis response and other behaviors in female golden hamsters. Physiol Behav 1977;19:223–37.

Mathews D, Edwards DA. Involvement of the ventromedial and anterior hypothalamic nuclei in the hormonal induction of receptivity in the female rat. Physiol Behav 1977;19:319–26.

McCarthy MM, Curran GH, Feder HH. Excitatry amino acid modulation of lordosis in the rat. Neurosci Lett 1991;126:94–7.

Meeker RB, Greenwood RS, Hayward JN. Glutamate receptors in the rat hypothalamus and pituitary. Endocrinology 1994;134:621–9.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 4th edition. San Diego: Academic Press; 1998.

Pfaff DW. Estrogens and brain function. New York: Springer-Verlag; 1980.

Pfaff DW, Keiner M. Atlas of estradiol-concentrating cells in the central nervous system of the female rat. J Comp Neurol 1973;151:121–58.

Pfaff DW, Sakuma Y. Facilitation of the lordosis reflex of female rats from the ventromedial nucleus of the hypothalamus. J Physiol 1979a;288:189–202.

Pfaff DW, Sakuma Y. Deficit in the lordosis reflex of female rats caused by lesions in the ventromedial nucleus of the hypothalamus. J Physiol 1979b;288:203–10.

Pfaus JG, Wilkins MF. A novel environment disrupts copulation in sexually naive but not experienced male rats: reversal with naloxone. Physiol Behav 1995;57:1045–9.

Pfaus JG, Marcangione C, Smith WJ, Manitt C, Abillamaa H. Differential induction of Fos in the female rat brain following different amounts of vaginocervical stimulation: modulation by steroid hormones. Brain Res 1996;741:314–30.

Pfaus JG, Smith WJ, Coopersmith CB. Appetitive and consummatory sexual behaviors of female rats in bilevel chambers: I. A correlational and factor analysis and the effects of ovarian hormones. Horm Behav 1999;35:224–40.

Pfaus JG, Smith WJ, Byrne N, Stephens G. Appetitive and consummatory sexual behaviors of female rats in bilevel chambers: II. Patterns of estrus termination following vaginocervical stimulation. Horm Behav 2000;37:96–107.

Puma C, Monmaur V, Sharif A, Monmaur P. Intraseptal infusion of selective and competitive e glutamate receptor agonist NMDA and antagonist D-2-amino-5-phosphonopentanoic acid: spectral implications for the physostigmine-induced hippocampal theta rhythm in urethane-anaesthesized rats. Exp Brain Res 1996;109:384–92.

Radecka H, Nakanishi J, Hirano A, Sugawara M, Umezawa Y. A method for evaluating chemical selectivity of agonists for glutamate receptor channels incorporated in liposomes based on an agonist-induced ion flux measured by ion-selective electrodes. J Pharm Biomed Anal 1999;19:205–16.

Rubin BS, Barfield RJ. Progesterone in the ventromedial hypothalamus facilitates estrous behavior in ovariectomized, estrogen-primed rats. Endocrinology 1983a;113:797–804.

Rubin BS, Barfield RJ. Induction of estrous behavior in ovariectomized rats by sequential replacement of estrogen and progesterone to the ventromedial hypothalamus. Neuroendocrinology 1983b;37:218–24.

Silveira MC, Graeff FG. Defense reaction elicited by microinjection of kainic acid into the medial hypothalamus of the rat: antagonism by a GABAA receptor agonist. Behav Neural Biol 1992;57:226–32.

Stubley-Weatherly L, Harding JW, Wright JW. Effects of discrete kainic acid-induced hippocampal lesions on spatial and contextual learning and memory in rats. Brain Res 1996;716:29–38.

Stumpf WE. Estrogen-neurons and estrogen-neuron systems in the periventricular brain. Am J Anat 1970;129:207–17.

Tanaka K, Watase K, Manabe T, Yamada K, Watanabe M, Takahashi K, et al. Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. Science 1997;276:1699–702.

Vornov JJ, Tasker RC, Park J. Neurotoxicity of acute glutamate transport blockade depends on coactivation of both NMDA and AMPA/kainate receptors in organotypic hippocampal cultures. Exp Neurol 1995;133:7–17.