Role of Ventromedial Hypothalamic Glutamate Receptors in the Regulation of Sexual Behavior of the Female Rat

Michaela Georgescu

A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfillment of the Requirements
for the Degree of Master of Arts at
Concordia University
Montreal, Quebec, Canada

September 2003

Your file Votre référence
ISBN: 0-612-83819-6
Our file Notre référence
ISBN: 0-612-83819-6

ABSTRACT

Role of Ventromedial Hypothalamic Glutamate Receptors in the Regulation of Sexual Behavior of the Female Rats'

Michaela Georgescu

Bilateral infusions of glutamate or kainate to the VMH have been shown previously to produce a rapid inhibition of lordosis induced by manual flank stimulation in estrogen (E)-primed female rats. The first experiment of this study examined whether glutamate and its specific receptor agonists AMPA, NMDA, and kainate, produce the same effect on lordosis, other proceptive, receptive, and defensive behaviors and ejaculations by males when administered to the VMH of E and progesterone (P)-primed female rats receiving olfactory, flank and vaginocervical stimulation (VCS) from sexually vigorous males. The obtained results suggest that glutamate specific receptor agonists, but not glutamate, have inhibitory effects on the entire battery of sexual behaviors. The second experiment investigated whether glutamate receptor antagonists AP-5, CNQX and DNQX have a facilitative effect on proceptivity and receptivity in E-, and E and P- primed females. Indeed, glutamate receptor antagonists had a facilitative effect on proceptive and receptive behaviors in both E-, and E and P- primed females, mirroring those of the agonists. Experiment 3 examined the effect of AP-5 on estrus termination induced by VCS with a lubricated glass rod. The hypothesis was that glutamate may be involved in the sexual inhibition observed during estrus termination, therefore administration of a neurochemical that would block its effects should counteract the effects of VCS. Contrary to this hypothesis, AP-5 heightened the behavioral effects of VCS by increasing defensive behaviors and reducing solicitation rates. Altogether, this data set suggests that VMH glutamate receptors play an inhibitory role on female sexual behavior, but that these receptors are not involved in estrus termination.

ACKNOWLEDGEMENTS

First and foremost, I would like to give thanks to my supervisor, Dr. Jim Pfaus, for his unrivaled approachability, friendly and agreeable guidance, teaching, and supervision, and great enthusiasm towards my project in his laboratory.

I would also like to thank Dr. Jane Stewart and Dr. Andrew Chapman for taking interest in my work by accepting to be part of my thesis committee, and also for being great role models regarding intellectual achievement.

My two-year experience in this laboratory would not have been the same without my good friends Veronica, Vicky and Amélie, who have entertained me greatly in the process, and helped me tremendously with surgeries and long testing hours, as well as on a personal level during not-so-great times.

Last but not least, I want to acknowledge Chris and my friends' words of encouragement, patience and listening; my mom for being more anxious and stressed than me about my lab work, thesis, and defense; my brother for punching in numbers for me, and Christine, with whom I have written almost my entire thesis...

TABLE OF CONTENTS

List of Figures vi

Introduction 1

Experiment 1

Introduction 10

Method 10

Results 16

Experiment 2

Introduction 25

Method 25

Results 26

Experiment 3

Introduction 37

Method 37

Results 41

Discussion 45

References 55

List of Figures

Figure 1. Placement data from subjects in Experiment 1.....14

Figure 2. Behavioral effects of glutamate 17

Figure 3. Behavioral effects of kainate 19

Figure 4. Behavioral effects of AMPA 21

Figure 5. Behavioral effects of NMDA 23

Figure 6. Placement data from subjects in Experiment 2 27

Figure 7. Behavioral effects of AP-5 28

Figure 8. Behavioral effects of CNQX 31

Figure 9. Behavioral effects of DNQX 34

Figure 10. Placement data from subjects in Experiment 3 40

Figure 11. Behavioral effects of AP-5 and VCS 42

The sexual behavior of the female rat consists of attractive, proceptive, and receptive behaviors that are largely regulated by two steroid hormones, estrogen (E) and progesterone (P) (Beach, 1976). A fundamental constituent of female reproductive behavior in rats is the lordosis reflex, which is the sexually receptive posture of the female rat, characterized by a dorsiflexion of the vertebral column. Lordosis is typically the sole aspect of sexual interaction that is addressed in studies pertaining to the neuroendocrinology of female sexual behavior (eg., Frye, Murphy and Platek, 2000; Luine, Wu, Hoffman, and Renner, 1999; Pfaff, Schwartz-Giblin, McCarthy, and Kow, 1994; Mathews and Edwards, 1977; Sakuma and Pfaff, 1983), despite the fact that female rats display a complex array of behaviors during sexual interaction (e.g., Erskine, 1989; McClintock, 1984, Pfaus, Smith, and Coopersmith, 1999). In fact, the sexual behaviors of female rats can be clustered in four types of behaviors: appetitive proceptive behaviors such as approach and solicitation, which allow females to be in contact with males; consummatory proceptive 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 every ejaculation (Pfaus et al., 1999).

E and P naturally secreted by the ovaries or artificially administered by subcutaneous injection induce receptivity and the full display of sexual behaviors.

Females used as subjects in experiments on the pharmacology of sexual behavior are typically ovariectomized to prevent impregnation and to allow experimental control over the estrus cycle with exogenous hormone replacement. A low degree of sexual receptivity is induced in ovariectomized females by subcutaneous injection of E, whereas subcutaneous injections of E followed by P 36-48 hours later result in full receptivity and a full complement of proceptive behaviors (Whalen, 1974).

A lordosis neural circuit that incorporates key evidence relating to hormonal input along with neural and spinal mechanisms has been presented by Pfaff and colleagues, who postulated four modules to explain how hormonal effects in limbic and hypothalamic neurons transform into behavioral effects (Pfaff, 1980; Pfaff, Schwartz-Giblin, McCarthy, and Kow, 1994). First, there is a spinal cord module that mediates sensorimotor reflexes. Pressure receptors on the flanks and hindquarters of the female rat evoke action potentials entering the spinal cord over dorsal roots L1, L2, L5, L6 and S1. Second, there is a hindbrain module that mediates activities across spinal levels: ascending fibers in the supraspinal loop travel to the brainstem and terminate in the medullary reticular formation, in the dorsocaudal lateral vestibular nucleus and in the MCG and in the peripenduncular region. Third, there is the midbrain module which provides the transition between neuroendocrine mechanisms of the hypothalamus and motor control of the hindbrain. Neurons from the MCG and the mesencephalic reticular formation send descending signals to the medullary reticulospinal neurons, which trigger activity in lumbar motor neurons that control the deep back muscles. These motor neurons allow muscle contractions that produce dorsiflexion of the vertebral column. Finally, there is a hypothalamic module that adds hormone dependence. At that point,

hypothalamic input enters the circuit when lordosis-related axons from E-sensitive neurons in particular descend from the VMH to the MCG. E, operating through receptors in the VMH, induces expression of the gene for P receptors, and P then binds to these receptors. Although this model illustrates well the general neurocircuitry behind lordosis, research indicates that at the micro level, the neural processes inherent to lordosis are far more complex, as Pfaff (1999) acknowledges.

The VMH is an important neural site in which both ovarian steroids act synergistically to regulate sexual behavior. The ventrolateral region of the VMH is particularly rich in E-concentrating cells (Stumpf, 1970; Pfaff and Keiner, 1973). Lesionning the VMH virtually eliminates the display of lordosis in rodents, (Mathews and Edwards, 1977; Pfaff and Sakuma, 1979a; Malsbury, Kow, and Pfaff, 1977), hamsters (Malsbury, Kow, and Pfaff, 1977) and guinea-pigs (Goy and Phoenix, 1963), whereas E implants to the VMH of ovariectomized females restore their ability to show lordosis (Dorner, Docke, and Moustafa, 1968; Rubin and Barfield, 1983b). Evidence supports the idea that the lesion-induced inhibition of lordosis in the VMH is attributed to the destruction of E-sensitive neurons (Pfaff and Sakuma, 1979a). Malsbury and colleagues (1977) proposed that VMH lesions may work to inhibit lordosis either by destroying cell bodies that are critical components of the hormone-sensitive circuitry controlling lordosis or by destroying axons of hormone-sensitive neurons passing through the VMH en route to other sites of the brain. On the other hand, P implants to the VMH of ovariectomized, E-primed rats further facilitates sexual behaviors (Barfield and Rubin, 1983). Moreover, electrical stimulation of this region results in a facilitation of lordosis in E-primed rats (Pfaff and Sakuma, 1979b). However, VMH neurons have relatively slow

firing rates (Dyer and Cross, 1972) which renders them unable to account for the fast lordosis reflex latencies (Pfaff and Sakuma, 1979b). Therefore, Pfaff and Sakuma concluded that it is unlikely that VMH neurons are directly involved in the lordosis reflex-arc, but that they control lordosis by exerting a tonic, hormone-sensitive bias on reflex-arcs in the midbrain or lower brainstem.

Whereas gonadal steroids induce sexual attractivity, proceptivity and receptivity, vaginocervical stimulation (VCS) of the female potentiates lordosis in the short-term, but induces a faster termination of the period of behavioral estrus (Hardy and DeBold, 1971). Female rodents can receive VCS either from multiple intromissions and ejaculations by the male, or from manual application of a lubricated glass rod (Pfaus, Kleopoulus, Mobbs, Gibbs, and Pfaff, 1993). Prior research established that VCS, distributed in a manner that mimics intromissions received by females during copulation, induces a behavioral pattern similar to that observed during estrus termination. This pattern is characterized by decreases in appetitive sexual responses and increases in rejection responses, prior to decline in the ability to lordose (Pfaus, Smith, Byrne, and Stephens, 2000). Subsequent to VCS administration in females, immunocytochemistry studies detected short- and long-term changes in many neural circuits that mediate appetitive and consummatory aspects of female sexual behavior. Thus far, induction of the immediately-early gene c-*fos* was detected following VCS within the VMH, mPOA, medial amygdala, lateral septum, ventral premammillary nuclei, lateral habenula, bed nucleus of the stria terminalis, paraventricular hypothalamic nucleus, arcuate, peripeduncular nuclei, striatum, the MCG and the cortex (Dudley, Rajendren, and Moss, 1996; Erskine, 1993; Pfaus et al., 1993; Pfaus et al., 1994; Pfaus et al., 1996; Polston and

Erskine, 1995; Rowe and Erskine, 1993; Tetel, Getzinger, and Blaustein, 1993, 1994; Tetel, Celentano, and Blaustein, 1994; Wersinger, Baum, and Erkstine, 1993). However, bilateral infusions of a sodium channel blocker to the VMH has been found to limit the ability of VCS to activate inhibitory circuits and thus preserved lordosis to a certain degree (Dobbek and Pfaus, 2001). This finding suggests that the VMH may be an important neural site which mediates the inhibition of sexual behavior observed during estrus termination. Therefore, the VMH does not only contain neural mechanisms that facilitate sexual behavior, but inhibitory ones that are activated during estrus termination as well.

Etgen and Karkanias (1993) postulated a model of facilitation of lordosis by interactions among ovarian steroids, norepinephrine (NE) and oxytocin (OXY) in the VMH, according to which OXY and NE act in synergy to increase the overall excitability of VMH neurons. The authors propose that the presence of E and P increases OXY receptor density and modifies their distribution within the VMH. OXY is then released from the posterior pituitary and perhaps other CNS sites and increases NE release in the VMH, possibly in response to VCS (Vincent and Etgen, 1993). The duration of the NE signal initiated by OXY may be prolonged if the female continues to receive somatosensory stimulation from the male because E also reduces the presynaptic inhibition of NE release mediated by α2-autoreceptors. Another possibility is that released NE interacts with α1-adrenoreceptors which increase in number following priming with E, promoting further release of OXY. Activation of hypothalamic α1-adrenoceptors increases neuronal excitability (Kim, Dudley, and Moss, 1988; Kow and Pfaff, 1987) and E potentiates these responses (Condon, Ronnekleiv, and Kelly, 1989).

Furthermore, α1-adrenoreceptors in the VMH mediate the facilitatory effect of NE on reproductive behavior in female rats (Etgen, 1990; Etgen, Ungar, and Petitti, 1992). Therefore, as OXY and NE act in concert to increase the overall excitability of VMH neurons, the probability that a female rat will exhibit lordosis when mounted by a male may increase dramatically in E-primed female rats. On the other hand, iontophoretically-applied dopamine decreases the electrical activity of VMH neurons (Chan, Dudley, and Moss, 1983). Steroid-dependent interactions between OXY and NE have also been reported in the medial preoptic area (mPOA), where OXY infusions increase NE of dialysates in E-primed ewes (Kendrick, Keverne, Hinton, and Goode, 1992).

Interestingly, there is also evidence of a lordosis-excitatory neural mechanism by an inhibitory neurotransmitter within the VMH. Gamma-amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the CNS. High concentrations of GABA have been detected in the lateral hypothalamus and the VMH (Kimura and Kuriyama, 1975; Tappaz, 1980; Frankfurt, Fuchs, and Wuttke, 1984), and increased levels of GABA in the VMH, the MCG, the medial basal hypothalamus and the VTA, but not the mPOA, facilitate lordosis (McCarthy, Malik, and Feder, 1990; McCarthy, Pfaff, and Schwartz-Giblin, 1990). GABA is synthesized in neurons from its precursor glutamate by the enzyme glutamic acid decarboxylase (GAD). McCarthy et al. (1994) found that infusions of GAD67 antisense mRNA to the VMH of OVX rats maintained on chronic E in silastic capsules resulted in a nearly complete inhibition of lordosis. Subsequently, the effect of E priming was analyzed on two forms of GAD, GAD65 and GAD67. There are two separate genes that encode the two forms of GAD, based on a 2 kDa difference in molecular weights. Furthermore, GAD67 is tonically active and synthesizes GABA for

non-vesicular release and/or metabolic purposes whereas GAD65 supplies the pool of GABA for vesicular release. It was found that E increased GAD65 and decreased GAD67 in the MPOA, but increased GAD67 and decreased GAD65 in the dorsal medial nucleus of the hypothalamus (McCarthy, Kaufman, Brooks, Pfaff, and Schwartz-Giblin, 1995). It is thus possible that E facilitates the synthesis of GAD in a population of GABA neurons that project to the VMH, and that release GABA during the period of behavioral estrus.

Given that a significant body of literature points to the presence of lordosis-facilitating neural mechanisms within the VMH, the effect of excitatory agents in this region was tested on lordosis (Kow, Harlan, Shivers, and Pfaff, 1985). However, microinfusions of glutamate, the primary excitatory amino acid in the brain, resulted in a powerful and rapid dose-related inhibition of lordosis induced by flank stimulation of ovariectomized females implanted with silastic capsules containing E. The ability to display lordosis recovered 20 minutes after the infusion. When the glutamate receptor agonist kainate was infused, similar results were obtained except that the effect was much longer lasting (about 4 days for full recovery). Electrophysiological follow-up of these experiments clarified that these agents excited VMH neurons and that this excitation was quick enough to account for the rapid inhibition of lordosis. These observations provided evidence of a lordosis-inhibiting neural mechanism by an excitatory agent within the VMH. Since that finding, no other experiments that examine the effect of glutamate in the VMH on sexual behavior have been published. However, Pfaus and Sabongui (1996) found that c-fos is induced in glutamate neurons in the VMH following VCS. Whether this finding means that glutamate release is involved in the sexual inhibition observed

following VCS, or that glutamate neurons are simply activated by VCS was not been determined.

The experiments presented in this thesis 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 (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), NMDA (*N*-methyl-D-aspartate) 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, Greenwood, and Hayward, 1994); each major glutamate subtype is present in all hypothalamic regions in the following approximate relative densities: NMDA > metabotropic glutamate receptor > kainate > AMPA.

In the present study, microinfusions of glutamate, or its selective receptor agonists or antagonists, were administered bilaterally to the VMH of ovariectomized, hormone-primed rats, and their effects were tested on a full battery of appetitive and consummatory sexual behaviors. The general hypothesis was that glutamate or its selective receptor agonists would decrease frequencies of appetitive and consummatory sexual behaviors and increase the incidence of defensive behaviors. Conversely, glutamate selective receptor antagonists would increase the occurrence of sexual behaviors and decrease the number of defensive behaviors, especially when females are

primed with E alone as opposed to E and P. Furthermore, it was hypothesized that a glutamate selective receptor antagonist would reverse the behavioral effects of estrus termination induced by VCS. Together, the data from these studies constitute the first comprehensive analysis of glutamate actions in the VMH on the sexual behavior of female rats.

EXPERIMENT 1

The goal of this experiment was to examine whether infusion of glutamate or its selective receptor agonists to the VMH disrupt or inhibit female sexual behavior. The study of Kow et al. (1985) was partially replicated: the effects of glutamate and kainate administered to the VMH were tested on female sexual behavior. However, Kow et al. tested the drugs in E-primed females and only the lordosis reflex was analyzed, whereas in the present study, drugs were administered to E- and P-primed females, and an entire battery of female sexual behaviors in response to stimulation from sexually vigorous males as opposed to manual flank stimulation was analyzed. Additionally, the number of male ejaculations was examined as a measure of overall female attractivity and copulatory competence.

Method

Subjects and procedure

Forty female and 40 male Long-Evans rats aged six weeks and weighing 200–250g, and 250–300g respectively, were obtained from Charles River Canada, Inc., St. Constant, Québec. Rats were housed in groups of four in hanging wire gang cages, but following cannulation, the females were housed individually in plastic cages (36 x 26 x 19 cm). Because rats are nocturnal animals, they were kept on a reversed 12 hour light/dark cycle, with lights off at 09:00, ensuring that they would be active during testing. The animals received commercial laboratory food and tap water at liberty. Room temperature was kept constant at 21°C.

All male and female rats received ten copulatory training sessions to ensure stable baseline rates of sexual behavior. During this forty-day copulatory training period,

ovariectomized females were tested every four days, and therefore received E 48 hours and P 4 hours prior to each copulatory session. This sexual experience was acquired in bi-level chambers, each session lasting 30 minutes. Following the tenth sexual trial, females were cannulated and allowed seven days of recovery from surgery. However, during this period of convalescence, females were hormonally primed twice (for 8 days) with E and P before testing occurred again. A baseline 30-minute copulatory session was then recorded immediately after a 1µl/side phosphate buffer (pH=7) infusion, during which each female was allowed to copulate with a sexually vigorous male in a bilevel chamber. All females were then randomly distributed to one of four groups: the first group received the lowest dose of glutamate (3.3mM); the second group received the medium dose (10mM), whereas the third received the highest (100mM). These doses are the same as those administered by Kow et al. (1985). The rats were redistributed to one of four groups prior to the administration of three selective glutamate receptor agonists, namely AMPA (0.3mM, 1mM, 2mM, Bell and Kalivas, 1996), NMDA (1nmol, 3.39nmol, 6.8nmol, Puma, Monmaur, Sharif and Monmaur, 1996) and kainate (0.469 mmol, 0.938 mmol, 1.17mmol; Kow et al., 1985). All drugs were obtained from Sigma. Once all the behavioral data were collected, females were sacrificed and their brains were removed to allow verification of cannulae placement. The inclusion criterion was that either one or both tips of the cannulae were found within the boundaries of the VMH or a millimeter dorsal to it.

Surgical, hormonal, drug administration and histological procedures

Ovariectomies. Females were ovariectomized bilaterally prior to copulatory training in order to prevent impregnation, and to allow full control over hormonal levels

and subsequently, the period of receptivity. The anesthesia consisted of a 4:3 mixture of ketamine hydrochloride (100mg/ml) to xylazine hydrochloride (20mg/ml) injected intraperitoneally in a volume of 0.8ml/kg. Ovaries were removed through lumbar incisions and a one-week period of recovery was allowed prior to copulatory training.

Hormonal injections. Full receptivity was induced in females by subcutaneous injections of estradiol benzoate (E), in a dosage of 10μg dissolved in 0.1 ml of sesame oil 48 hours and 500μg of P in 0.1 ml of sesame oil four hours before each test.

Cannulations. Prior to cannulation, females were anesthetized with sodium pentobarbital (65mg/kg) injected intraperitoneally. Females were then cannulated bilaterally to the VMH using a stereotaxic instrument and double-barrel 26-gauge guide cannulae. The cannulation coordinates used to attain the VMH were 1mm lateral to the midline on either side of Bregma, 0mm anterior to Bregma, and 8.5mm ventral to Dura, at a 5-degree elevation angle. The tip of the cannula guide ended 1mm above the desired target area. Cannula blockers were cut so that they would protrude 0.5mm from the guide cannula. The 33-gauge infusion cannulae were cut to 1mm longer than the guide cannula. Guide cannulae, cannulae blockers, injection cannulae and dust caps were obtained from Plastic One. Females were given seven days of *post*-surgical recovery before testing began.

Infusions. All drugs were infused at a rate of 1μl/minute for one minute using an infusion pump (Harvard Apparatus, Pump 22). A total volume of 1μl was infused per side. The infusor was left in for another full minute and a half to ensure full absorption. The desired concentration of glutamate, AMPA, NMDA and kainate were obtained by

diluting the drugs into a phosphate buffer (pH = 7). In the case of saline infusions, a 0.1M phosphate buffer with a neutral pH of 7 was infused in a volume of 1μl/side.

Perfusions and histology. Once behavioral data were collected, females were sacrificed in order to verify proper cannulae placement. They were injected with 1ml of sodium pentobarbital and perfused intracardially using a 50ml syringe filled with ice-cold phosphate buffer saline followed by 50ml of 4% paraformaldehyde in 0.1M phosphate buffer. The brains were placed in a 4% paraformaldehyde solution for 4 hours, and then into a 30% sucrose solution. Once removed from this solution, the brains were frozen using dry ice and sliced into coronal sections using a cryostat. These sections were mounted on gel-coated slides, stained in cresyl violet, cover slipped, and examined under a microscope to confirm cannulae placements. The exclusion criteria was set such that animals with both guide cannulae ending outside of the boundaries of the VMH or more than 1mm dorsal to it were excluded from the study (N = 4), leaving data solely from subjects that had correct unilateral (N = 8) or bilateral (N = 21) cannulations to the VMH included in the analyses. Preliminary statistical investigations were conducted to assess whether data from animals that died (N = 7) during the course of the experiment and from whom cannulae placement data were not collected should be excluded from the study. The results obtained from these analyses were almost identical to the results obtained from analyses excluding data from such subjects. Consequently, the data from these animals were included in the final statistical analyses in order to increase N and subsequently, the power of the design. Cannulation placement data from subjects included in the statistical analyses of Experiment 1 are shown in Figure 1. It is important to note that subjects in Experiment 1, as opposed to those in Experiments 2 and 3, were

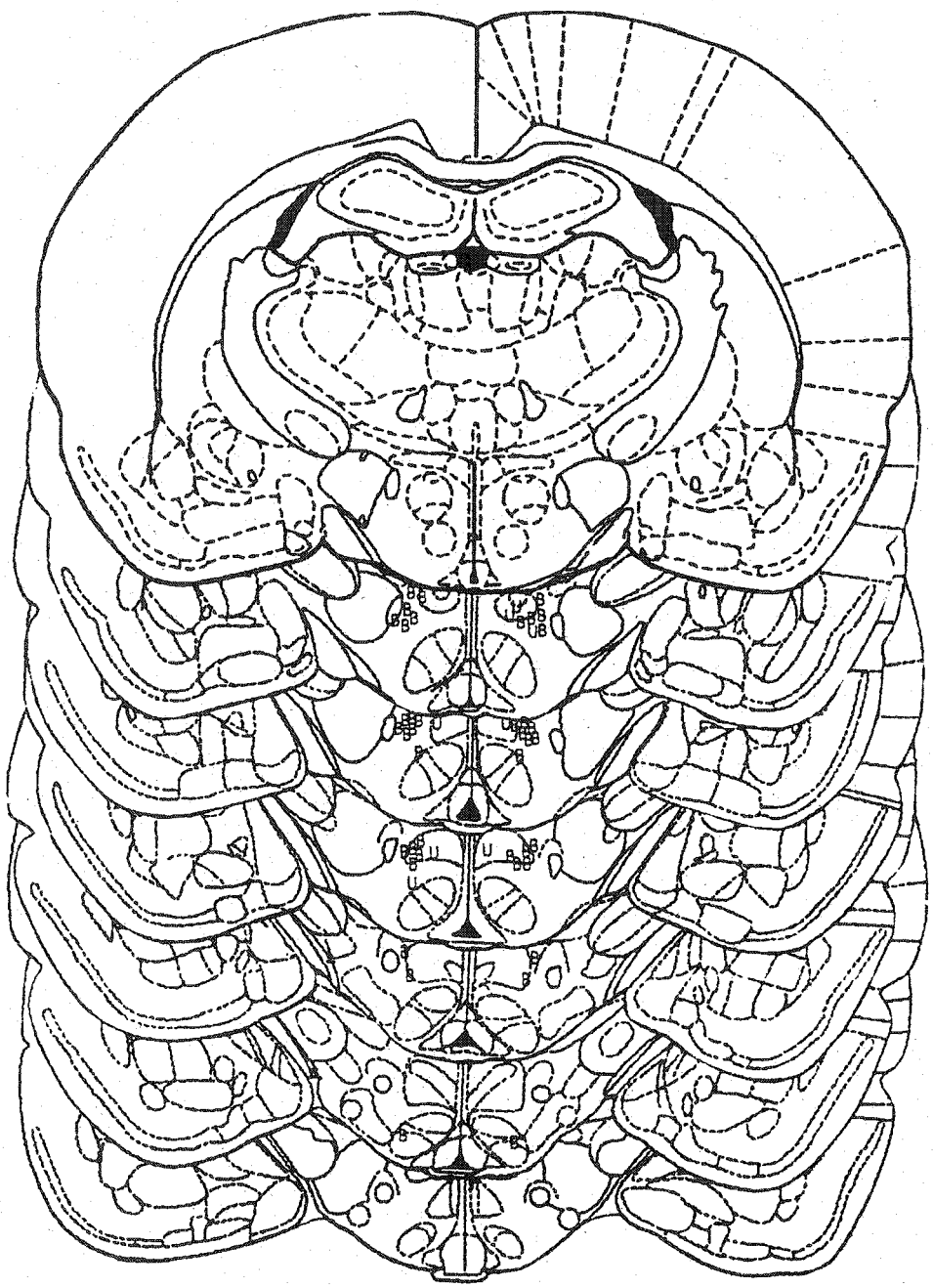

Figure 1. Placement data for subjects in Experiment 1. Correct bilateral cannulations are indicated by the letter "B," whereas correct unilateral cannulations are indicated by the letter "U."

perfused without cannulae blockers in the cannulae, therefore correct placements in Experiment 1 appear 0.5mm dorsal to placements from subjects in Experiment 2 and 3.

Behavioral analyses

Testing sessions were videotaped using a camcorder; the footage was viewed using a VCR and a television, and scored using a computerized event recorder customized for female sexual behavior (Cabilio, 1996). Data from the first ejaculatory series (namely from the introduction of the female into the bilevel chamber to the first ejaculation of the male or a 30-minute period when ejaculation did not occur) were analyzed, contrasting saline behavioral results with behavioral results obtained under the influence of each dosage of the drug. The frequency of solicitations (characterized by a head-wise orientation of the female towards the male followed by a quick runaway from the male), hops and darts (H&D), pacing (or level changing (LC), occurring when the female moves from one level of the bilevel chamber to the other), and rejection responses (including boxing, fighting, kicking and prone defensiveness, characterized by the female lying on her back) were included in the analyses. Lordosis was analyzed from two perspectives: lordosis reflex magnitude (LM, on a 1 to 3 scale), and lordosis quotient (LQ, lordosis:mount ratio). LM and LQ were calculated as in Hardy and DeBold (1971). Finally, the number of male ejaculations during the half hour session was also recorded.

Statistical analyses

Separate 2 (treatment: drug vs. saline) x 3 (dose: small, medium, large) analyses of variance (ANOVAs) with dose as a between measures variable and treatment as a repeated measures variable were performed on all sexual behaviors, namely solicitations, H&D, pacing, LQs, LMs, defensive behaviors, and male ejaculations. In each case, the

saline trial served as the baseline measure to which drug measures were compared. The saline trials were conducted prior to the drug trials. Tukey *post hoc* comparisons of means analyses were conducted when statistical significance was detected by the ANOVAs to analyze differences between the means of each group. Only the significant main effects or interaction are reported.

Results

Behavioral observations

Following infusions of glutamate and AMPA, the female rats did not require special or cautious handling; no behaviors related to hyperactivity, for example, were observed. However, a small number of females were difficult to handle during and following infusions of kainate and NMDA. Hyperactivity-related behaviors were observed: some of the females spent large portions of the testing session engaged in level changing or chewing on the walls or metal floor grids of the bilevel chamber.

Glutamate

Figure 2 shows the effects of infusions of saline and 3 different doses of glutamate in the VMH on the number of solicitations, H&D, LC, LQ, LM, defensive behaviors and male ejaculations. Overall, infusions of the small and medium dose of glutamate increased the number of solicitations, whereas infusion of the large dose decreased solicitation. Furthermore, relative to saline, glutamate infusions decreased the number of hops and darts, slightly increased pacing, had no effect on the LQ and LM, increased the number of defensive behaviors when the medium dose was infused and increased the number of ejaculations. However, the ANOVA detected only a significant main effect of treatment on the number of H&D, $F(1, 24) = 10.43$, $p < 0.01$. *Post hoc*

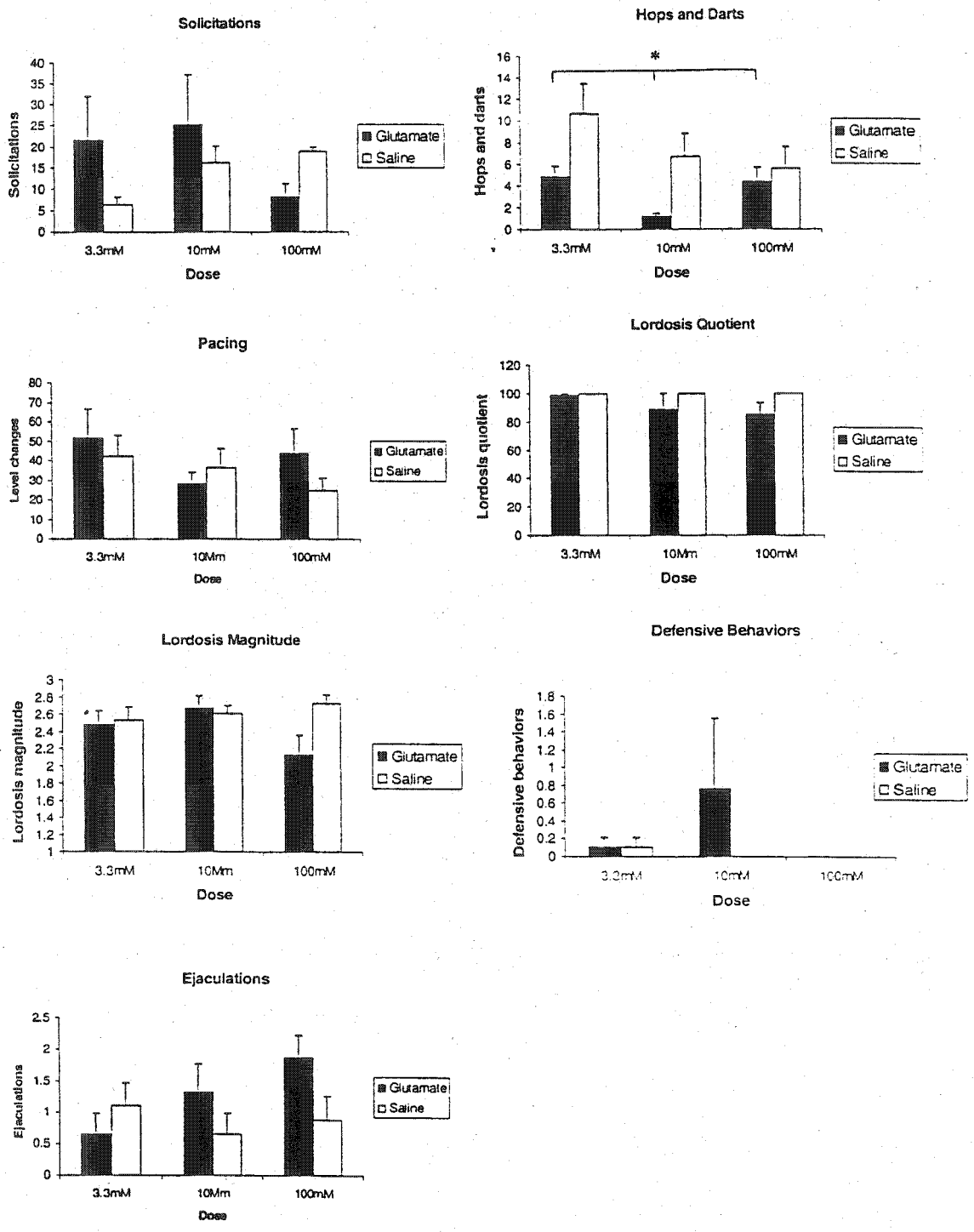

Figure 2. The effect of 3 doses of glutamate or saline infusions on the mean number of solicitations, hops and darts, level changes, mean lordosis magnitude, mean lordosis quotient, mean number of defensive behaviors and male ejaculations. Error bars represent standard errors. (* = $p < .05$, effect of dose: 3.3mM, 10mM vs 100mM, between subjects)

comparisons revealed that animals engaged in less H&D when infused with glutamate than when infused with saline. The infusions of glutamate had no significant effects on any other measures, namely on solicitation, pacing, LM, LQ, defensive behavior, and male ejaculations.

Kainate

Figure 3 shows the effects of infusions of saline and 3 different doses of kainate on the number of solicitations, H&D, pacing, LQ, LM, defensive behaviors and male ejaculations. Infusions of kainate produced decreases in solicitation, LQs and LMs, dose-related decreases in H&D, increases in pacing and defensive behaviors, but had no effect on male ejaculations.

Solicitations. The ANOVA detected a significant main effect of treatment, $F(1, 23) = 4.90$, $p < 0.05$. *Post hoc* analyses revealed that animals in the drug group solicited sex significantly less than animals in the saline group.

Hops and darts. The ANOVA detected a significant interaction between dose and treatment, $F(2, 23) = 4.23$, $p < 0.05$. Kainate produced a dose-related decrease in the number of H&D. *Post hoc* analyses revealed that females infused with the largest dose of kainate engaged in significantly less H&D than did animals infused with saline.

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

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 infusions resulted in lower LQ scores than did saline infusions.

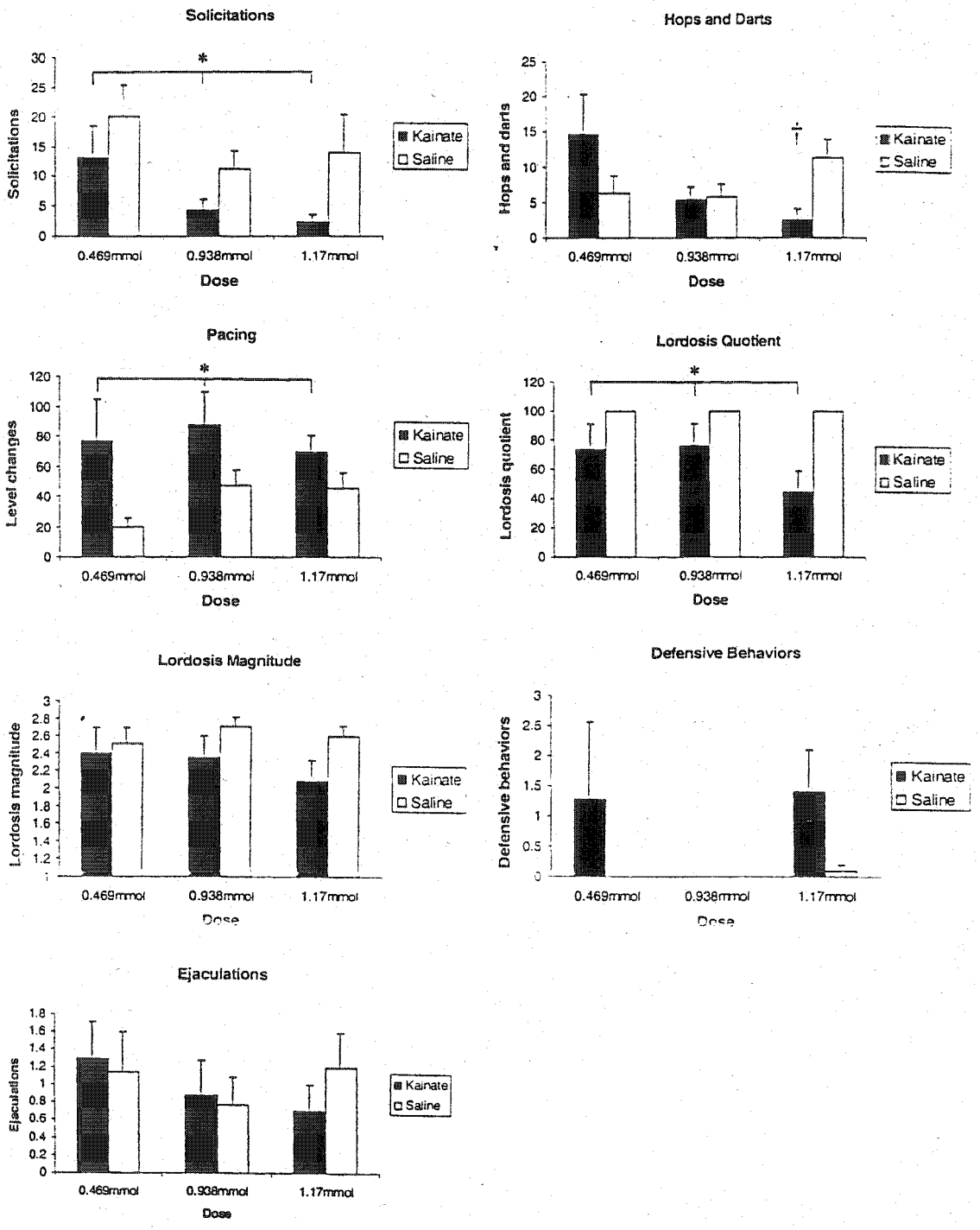

Figure 3. The effect of 3 doses of kainate or saline infusions on the mean number of solicitations, hops and darts, level changes, mean lordosis magnitude, mean lordosis quotient, mean number of defensive behaviors and male ejaculations. Error bars represent standard errors. (* = $p < .05$, effect of dose: 0.469mmol vs 0.938mmol vs 1.17mmol, between subjects; † = $p < .05$, effect of treatment: kainate vs saline, within subjects)

Lordosis magnitude, defensive behaviors, ejaculations. No significant main effects of dose or treatment were detected. The interaction between these two variables did not reach the required level of statistical significance either.

AMPA

Figure 4 shows the effects of infusions of saline and 3 different doses of AMPA on the number of solicitations, H&D, pacing, LQ, LM, defensive behaviors and male ejaculations. Overall, infusions of AMPA decreased the number of solicitations, H&D, increased pacing, decreased LQs and LMs, increased the number of defensive behaviors following infusion of the small dose only, and had no effect on the number of ejaculations.

Solicitations. The ANOVA detected a significant main effect of treatment, $F(1, 25) = 14.99$, $p < 0.001$, *post hoc* analyses revealing that animals infused with AMPA solicited significantly less than animals infused with saline.

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 infused with AMPA engaged in significantly less H&D than animals infused with saline.

Lordosis quotient. A significant main effect of treatment was detected, $F(1, 25) = 13.91$, $p < 0.001$. *Post hoc* analyses revealed that when animals were infused with AMPA, they obtained significantly lower LQs than when they were infused with saline.

Lordosis magnitude. The ANOVA detected a significant main effect of treatment, $F(1, 19) = 15.97$, $p < 0.001$. *Post hoc* analyses revealed that AMPA infusions resulted in lower LMs than saline infusions.

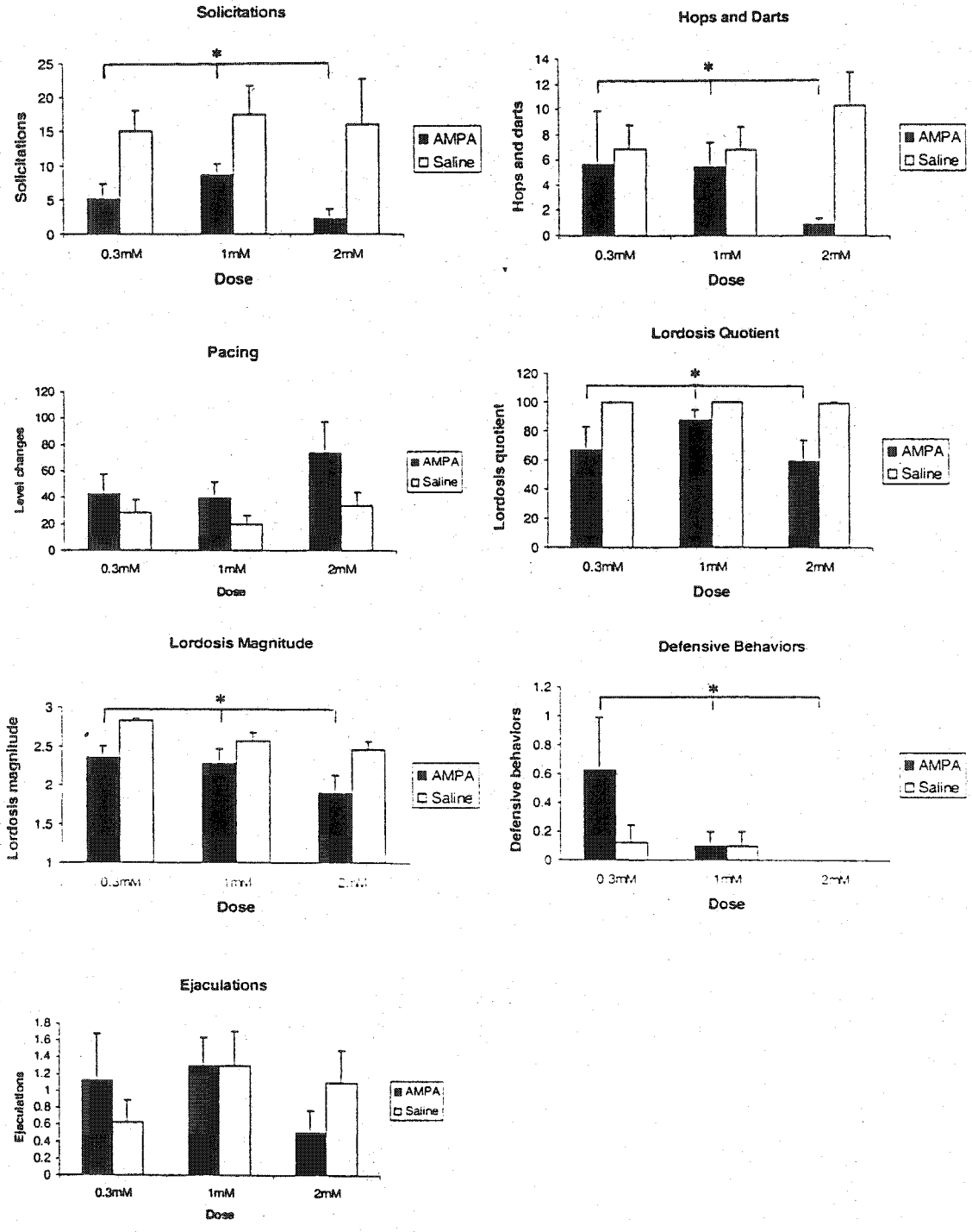

Figure 4. The effect of 3 doses of AMPA or saline infusions on the mean number of solicitations, hops and darts, level changes, mean lordosis magnitude, mean lordosis quotient, mean number of defensive behaviors and male ejaculations. Error bars represent standard errors. (* = $p < .05$, effect of dose: 0.3mM vs 1mM vs 2mM, between subjects)

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 small dose of AMPA engaged in significantly more defensive behaviors than animals that received the medium or high dose of AMPA.

Pacing and ejaculations. The ANOVAs failed to detect any significant main effects or interactions between dose and treatment on these behaviors.

NMDA

Figure 5 shows the effects of infusions of saline and 3 different doses of NMDA on the number of solicitations, H&D, pacing, LQ, LM, defensive behaviors and male ejaculations. NMDA infusions increased pacing and the number of defensive behaviors, and decreased LQs, LMs, and the number of male ejaculations, but had no effect on the number of solicitations and H&D.

Pacing. The ANOVA detected a significant main effect of treatment, $F(1, 25) = 16.32$, $p < 0.001$. *Post hoc* analyses revealed that animals infused with NMDA level changed significantly more than animals infused with saline.

Lordosis quotient. A significant main effect of treatment was detected, $F(1, 25) = 11.84$, $p < 0.01$. *Post hoc* analyses revealed that NMDA infusions resulted in significantly lower LQs than saline infusions.

Lordosis magnitude. The ANOVA detected a significant main effect of dose, $F(2, 23) = 5.71$, $p < 0.01$. *Post hoc* analyses revealed that infusions with the high dose of NMDA resulted in larger LM scores than infusions with the small and medium doses.

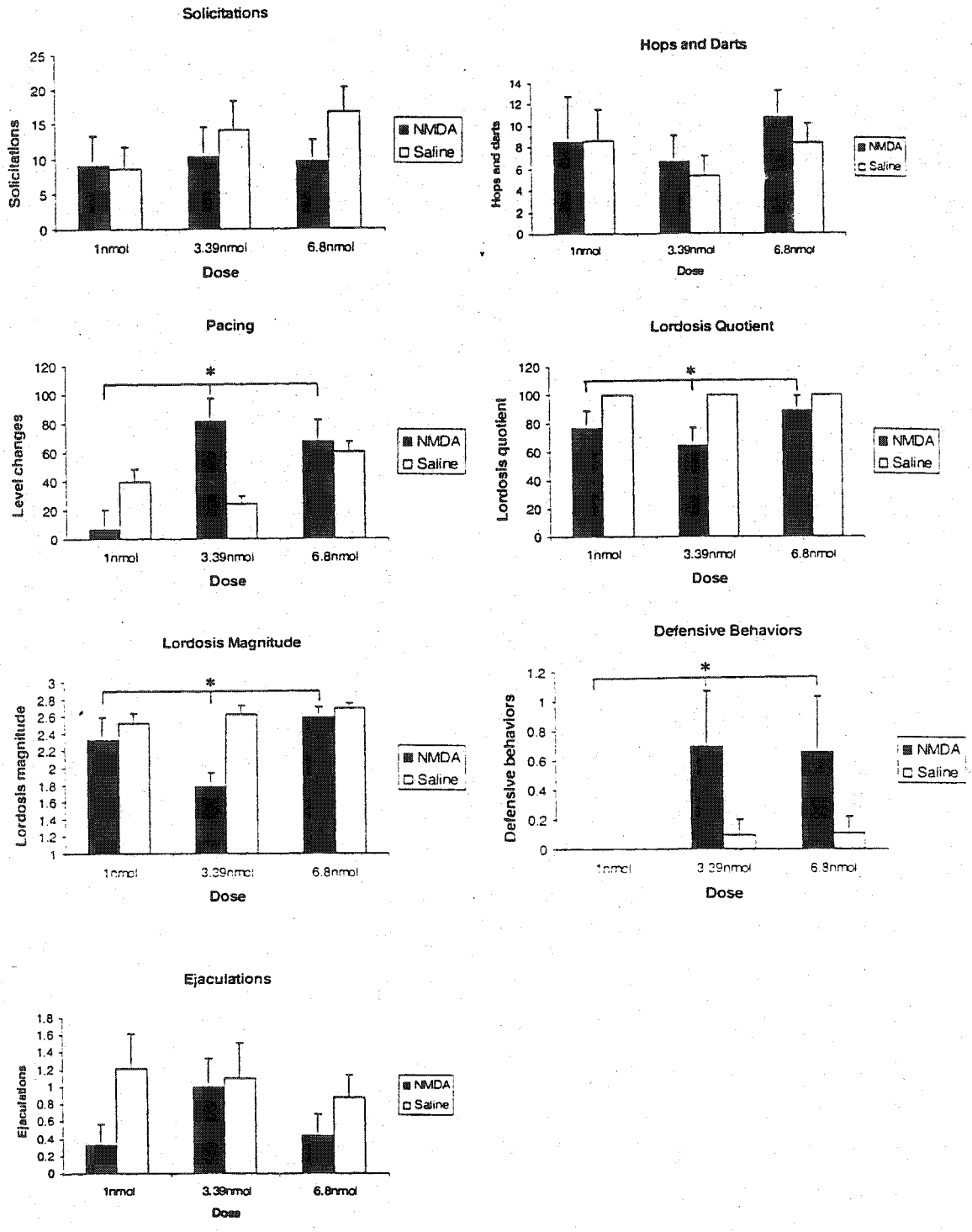

Figure 5. The effect of 3 doses of NMDA or saline infusions on the mean number of solicitations, hops and darts, level changes, mean lordosis magnitude, mean lordosis quotient, mean number of defensive behaviors and male ejaculations. Error bars represent standard errors. (* = $p < .05$, effect of dose: 1nmol vs 3.39nmol vs 6.8nmol, between subjects)

Furthermore, a significant main effect of treatment was detected, $F(1, 23) = 7.89$, $p < 0.01$. *Post hoc* analyses revealed that rats infused with NMDA obtained smaller LM scores than rats infused with saline.

Defensive behaviors. The ANOVA detected a significant main effect of treatment, $F(1, 25) = 4.21$, $p < 0.01$. *Post hoc* analyses further revealed that animals infused with NMDA displayed more defensive behaviors than animals infused with saline.

Solicitations, hops and darts, ejaculations. No significant main effects or interactions between dose and treatment were detected by the ANOVAs.

EXPERIMENT 2

When glutamate or its selective receptor agonists were infused in the VMH, some inhibitory effects were observed on the sexual behavior of E- and P-primed females. The purpose of the second experiment was to assess whether the effects of selective competitive antagonists of the NMDA, AMPA and kainate receptors (2-amino-5-phosphonopentanoic acid (AP-5) for the NMDA receptor, 6,7-dinitroquinoxaline-2,3-dione (DNQX) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) for the AMPA/kainate receptors) tested on E- or E+P-primed females following infusion into the VMH might facilitate sexual behavior.

Method

Subjects and surgical procedures

Nineteen females and 20 males were tested in this experiment. They were housed in the same conditions as animals from Experiment 1. The sexual training trials, surgical, and histological procedures were performed in the same manner as those in the Experiment 1.

Experimental procedure

Following the period of recovery from the cannulation procedure, the 20 females were randomly distributed to one of two groups, namely those that would be primed with E-alone, and those that would be primed with E+P as in the first experiment. Animals primed with E received their injection subcutaneously 4 hours prior to behavioral testing. A baseline copulatory session following infusion of a phosphate buffer was then recorded for both groups. All rats were administered 1μl/side of each one of the 2 doses of AP-5, CNQX, and DNQX. The dosages used were the following: for

AP-5, the small dose (Dose 1) was 10mg/ml, whereas the large dose (Dose 2) was 20mg/1ml (Park, Bari, Jey, Anderson, Spealman, Rowlett, and Pierce, 2002; DiCiano and Everitt, 2001); for CNQX, 0.005mM (small dose or Dose1) and 0.6M (large dose or Dose 2), (Alvarez & Ruarte, 2001; Park et al., 2002); for DNQX, 0.397mMol (small dose or Dose 1) and 1.98mMol (small dose or Dose 2), (Roullet, Sargolini, Oliverio, and Mele, 2001). All testing sessions took place in bilevel chambers. Once behavioral data was obtained, females were sacrificed, and their brains were removed to verify cannula placements, which are shown in Figure 6. Out of 20 placements, 9 were bilateral, 9 were unilateral, one was unknown and one was incorrect and therefore excluded from the analyses.

Statistical analyses

Separate 2 (hormone: E-alone or E+P) x 3 (dose: saline or small dose or large dose) ANOVAs with repeated measures on the second factor were performed on all sexual behaviors. When significant main effects or interactions were detected, Tukey *post hoc* analyses were performed to further elucidate where the differences lay. Only the significant main effects or interactions between these variables are reported.

Results

Behavioral observations

No unusual behaviors were observed following infusion of AP-5, CNQX or DNQX; animals were easy to handle.

AP-5

Figure 7 shows the effects of infusions of saline and 2 different doses of AP-5 administered to the VMH of E-alone or E+P-primed female rats, on the number of

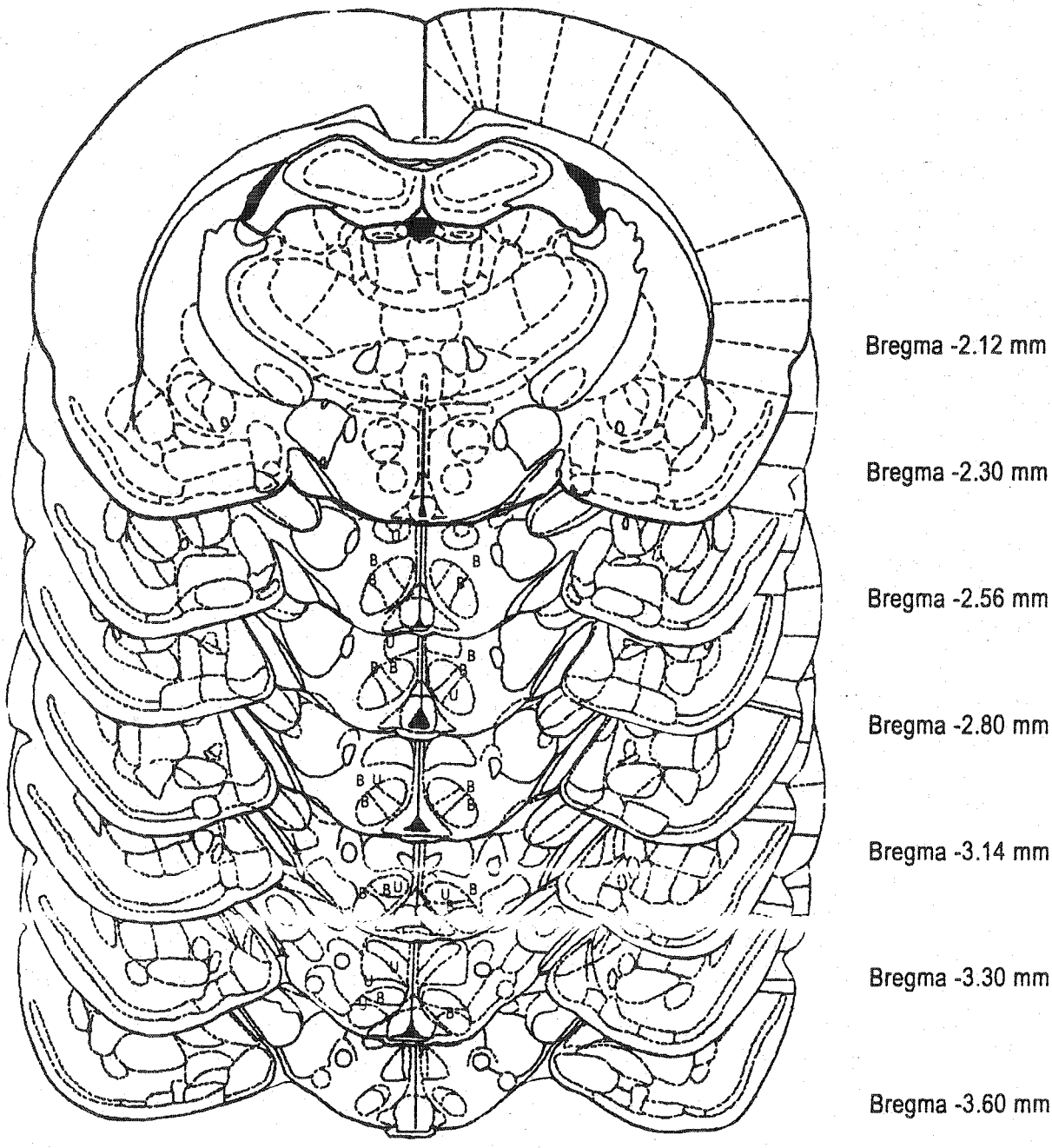

Figure 6. Placement data for subjects in Experiment 2. Correct bilateral cannulations are indicated by the letter "B," whereas correct unilateral cannulations are indicated by the letter "U."

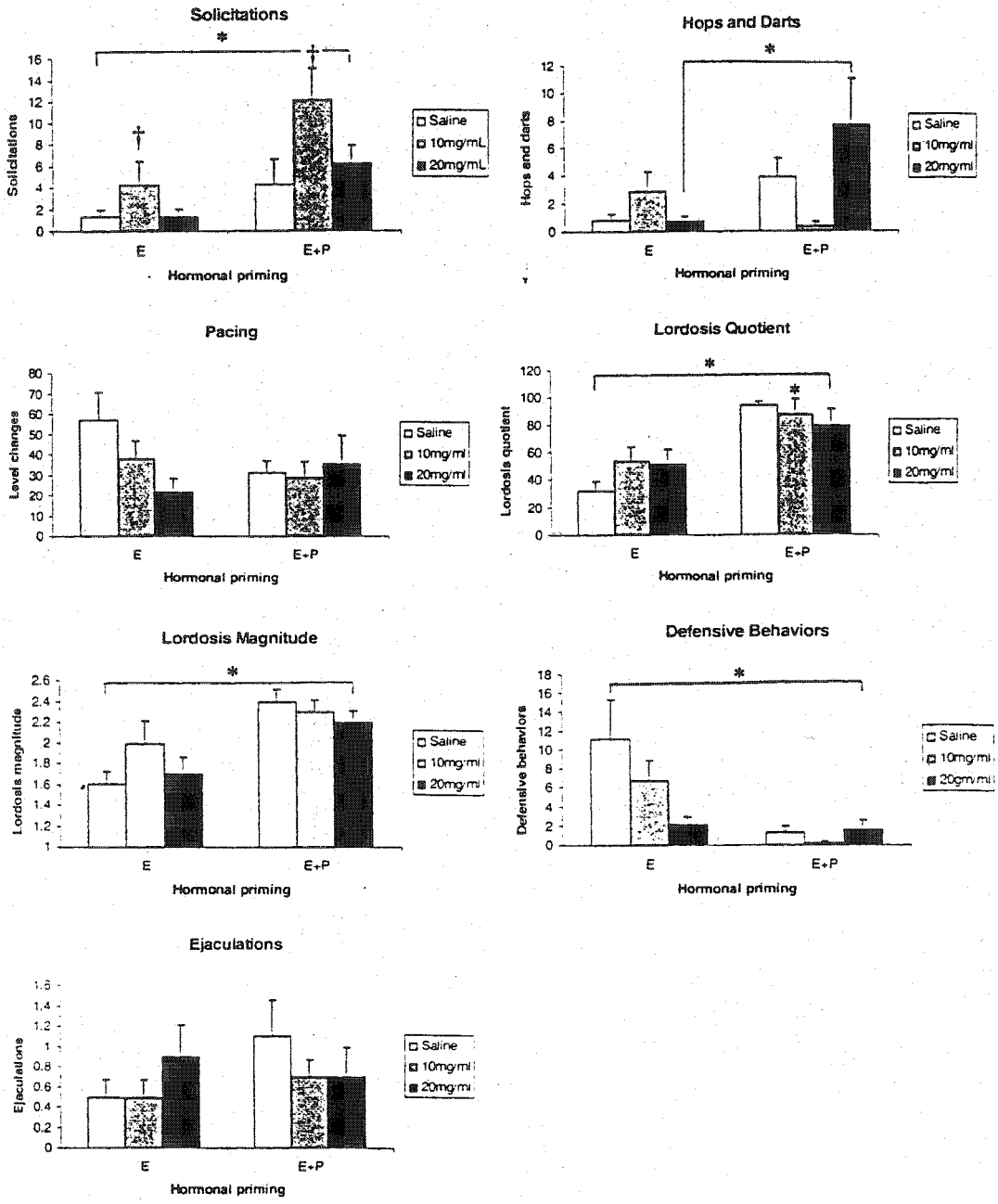

Figure 7. The effect of 2 doses of AP-5 or saline infusions on the mean number of solicitations, hops and darts, level changes, mean lordosis magnitude, mean lordosis quotient, mean number of defensive behaviors and male ejaculations. Error bars represent standard errors. († = $p < .05$, effect of dose: saline vs 10mg/ml, 20mg/ml, within subjects; * = $p < .05$, effect of hormonal priming: E vs E+P, between subjects)

solicitations, H&D, pacing, LQ, LM, defensive behaviors and male ejaculations. In E-alone-primed females, infusions of AP-5 relative to saline increased LQs, LMs and the number of male ejaculations, and decreased the number of defensive behaviors and LCs, however had no substantial effect on the number of solicitations and H&D. In E+P-primed females, only the number of solicitations and H&D appear to have increased following infusions of AP-5.

Solicitations. A significant main effect of hormone was detected, $F(1, 17) = 17.84$, $p < 0.001$. The *post hoc* analysis revealed that rats primed with E+P solicited sex more than rats primed with E-alone. The ANOVA also detected a significant main effect of dose, $F(2, 34) = 3.51$, $p < 0.05$. *Post hoc* analyses revealed that rats who received infusions of the small dose of AP-5 solicited sex more than rats who received saline infusions.

Hops and darts. The ANOVA detected a significant interaction between hormone and dose, $F(2, 34) = 4.78$, $p < 0.05$. *Post hoc* analyses revealed that females primed with E+P and who received the large dose of AP-5 engaged in significantly more H&D than animals that were also primed with E+P but received the small dose of AP-5 and animals that were primed with E-alone and who received saline infusions or the large dose of AP-5.

Lordosis quotient. A significant main effect of hormone was detected, $F(1, 17) = 15.85$, $p < 0.001$. *Post hoc* analyses further revealed that animals primed with E-alone had lower LQs than animals primed with E+P. Moreover, there was a significant interaction between hormone and dose, $F(2, 34) = 3.34$, $p < 0.05$. The Tukey *post hoc* revealed that following infusion of the small dose, animals primed with E+P had

significantly larger LQs than animals primed with E-alone. Furthermore, animals primed with E+P obtained significantly larger LQs than animals primed with E-alone following saline infusions.

Lordosis magnitude. The ANOVA detected a significant main effect of hormone, $F(1, 14) = 13.54$, $p < 0.005$. *Post hoc* analyses revealed that animals primed with E+P had larger LMs than animals primed with E-alone.

Defensive behaviors. Hormonal manipulation had a significant main effect on the display of overt defensive behaviors, $F(1, 17) = 15.26$, $p < 0.01$, *post hoc* analyses revealing that subjects primed with E-alone engaged in significantly more defensive behaviors than subjects primed with E+P.

Pacing and ejaculations. ANOVAs failed to detect any significant main effects or interactions between dose and hormone on LCs or ejaculations.

CNQX

Figure 8 shows the effects of infusions of saline and 2 different doses of CNQX administered to the VMH of E-alone or E+P-primed female rats, on the number of solicitations, H&D, pacing, LQ, LM, defensive behaviors and male ejaculations. In E-alone-primed females, infusions of CNQX increased the number of solicitations, H&D, LQs and LMs, and the number of ejaculations by the males relative to saline. In E+P-primed females, the number of solicitations, LCs and ejaculations by the males were increased following infusions of AP-5.

Solicitations. The ANOVA revealed a significant main effect of hormone, $F(1, 17) = 6.05$, $p < 0.05$. *Post hoc* analyses revealed that females primed with E+P solicited sex more than females primed with E-alone. The ANOVA further detected a significant

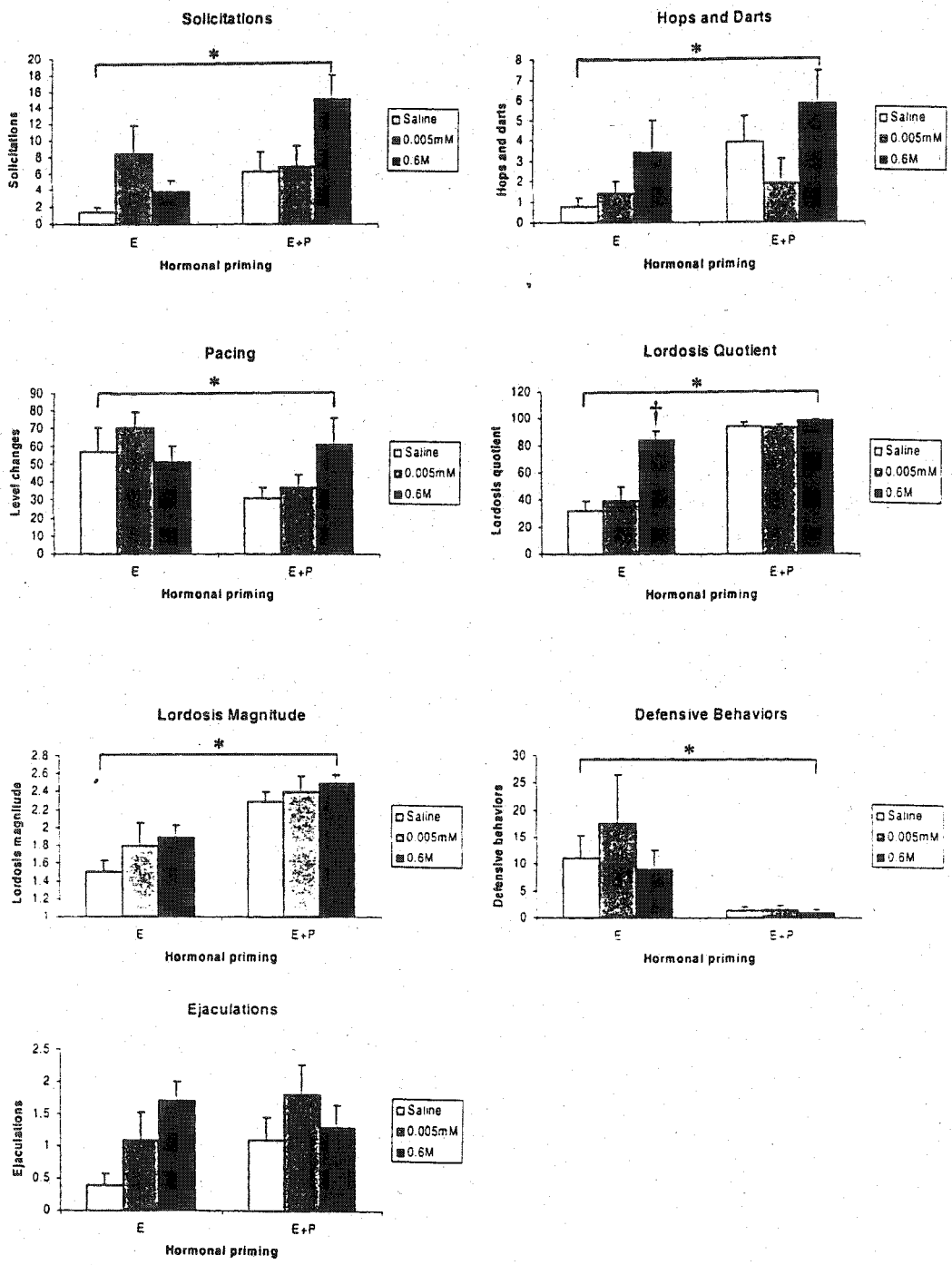

Figure 8. The effect of 2 doses of CNQX or saline infusions on the mean number of solicitations, hops and darts, level changes, mean lordosis magnitude, mean lordosis quotient, mean number of defensive behaviors and male ejaculations. Error bars represent standard errors. († = $p < .05$, effect of dose: saline vs 0.005mM vs 0.6M, within subjects; * = $p < .05$, effect of hormonal priming: E vs E+P, between subjects)

dose x hormone interaction, $F(2, 34) = 3.52$, $p < 0.05$. *Post hoc* analyses revealed that E+P-primed females who received the large dose of CNQX solicited sex more than E-primed females who were infused with saline or the high dose of CNQX.

Hops and darts. The ANOVA detected a significant main effect of hormone, $F(1, 17) = 5.13$, $p < 0.05$, *post hoc* analyses revealing that hormonal priming with E+P resulted in significantly more H&D than priming with E-alone did.

Pacing. The ANOVA detected a significant main effect of hormone, $F(1, 17) = 4.55$, $p < 0.05$. *Post hoc* analyses revealed that females primed with E-alone level changed more than females primed with E+P.

Lordosis quotient. The ANOVA detected a significant main effect of hormone, $F(1, 17) = 86.27$, $p < 0.000\ 1$, *post hoc* analyses revealing that females primed with E+P had significantly higher LQs than females primed with E-alone. A significant main effect of dose was also detected, $F(2, 34) = 11.90$, $p < 0.001$. *Post hoc* analyses revealed that females who received the large dose of CNQX obtained significantly larger LQs than females who received the small dose of CNQX or saline infusions. Finally, the ANOVA detected a significant interaction between hormone and dose, $F(2, 34) = 8.05$, $p < 0.01$, *post hoc* analyses revealing that E-primed females who received the high dose of CNQX obtained significantly larger LQs than E-primed females who received the small dose or saline; also, E-primed females who received saline or the small dose of CNQX obtained significantly smaller LQs than E+P-primed females who underwent the same treatment.

Lordosis magnitude. The ANOVA detected a significant main effect of hormone, $F(1, 14) = 23.03$, $p < 0.001$. *Post hoc* analyses revealed that rats primed with E+P obtained larger LMs than rats primed with E-alone.

Defensive behaviors. The ANOVA detected a significant main effect of hormone, $F(1, 17) = 7.93$, $p < 0.01$, *post hoc* analyses revealing that rats primed with E displayed significantly more defensive behaviors than animals primed with E+P.

Ejaculations. No significant main effects of hormone or dose were detected by the ANOVA. The interaction between hormone and dose was also non significant.

DNQX

Figure 9 shows the effects of infusions of saline and 2 different doses of DNQX administered to the VMH of E-alone or E+P-primed female rats, on the number of solicitations, H&D, pacing, LQ, LM, defensive behaviors and male ejaculations. In E-alone-primed females, infusions of DNQX increased the number of solicitations, H&D, increased LQs and LMs and the number of ejaculations by the males, however the drug decreased the number of LCs and defensive behaviors, relative to saline. In E+P-primed females, infusions of DNQX increased the number of solicitations and decreased the number of LCs.

Solicitations. The ANOVA detected a significant main effect of hormone, $F(1, 15) = 11.59$, $p < 0.01$. *Post hoc* analyses revealed that animals treated with E+P solicited sex significantly more than animals treated with E-alone. A significant main effect of dose was also detected, $F(2, 30) = 6.41$, $p < 0.01$, *post hoc* analyses revealing that animals infused with the large dose of DNQX solicited sex significantly more than animals infused with saline.

Pacing. The ANOVA detected a significant main effect of hormone, $F(1, 15) = 6.50$, $p < 0.05$, *post hoc* analyses revealing that animals primed with E-alone level changed significantly more than animals primed with E+P. A significant main effect of

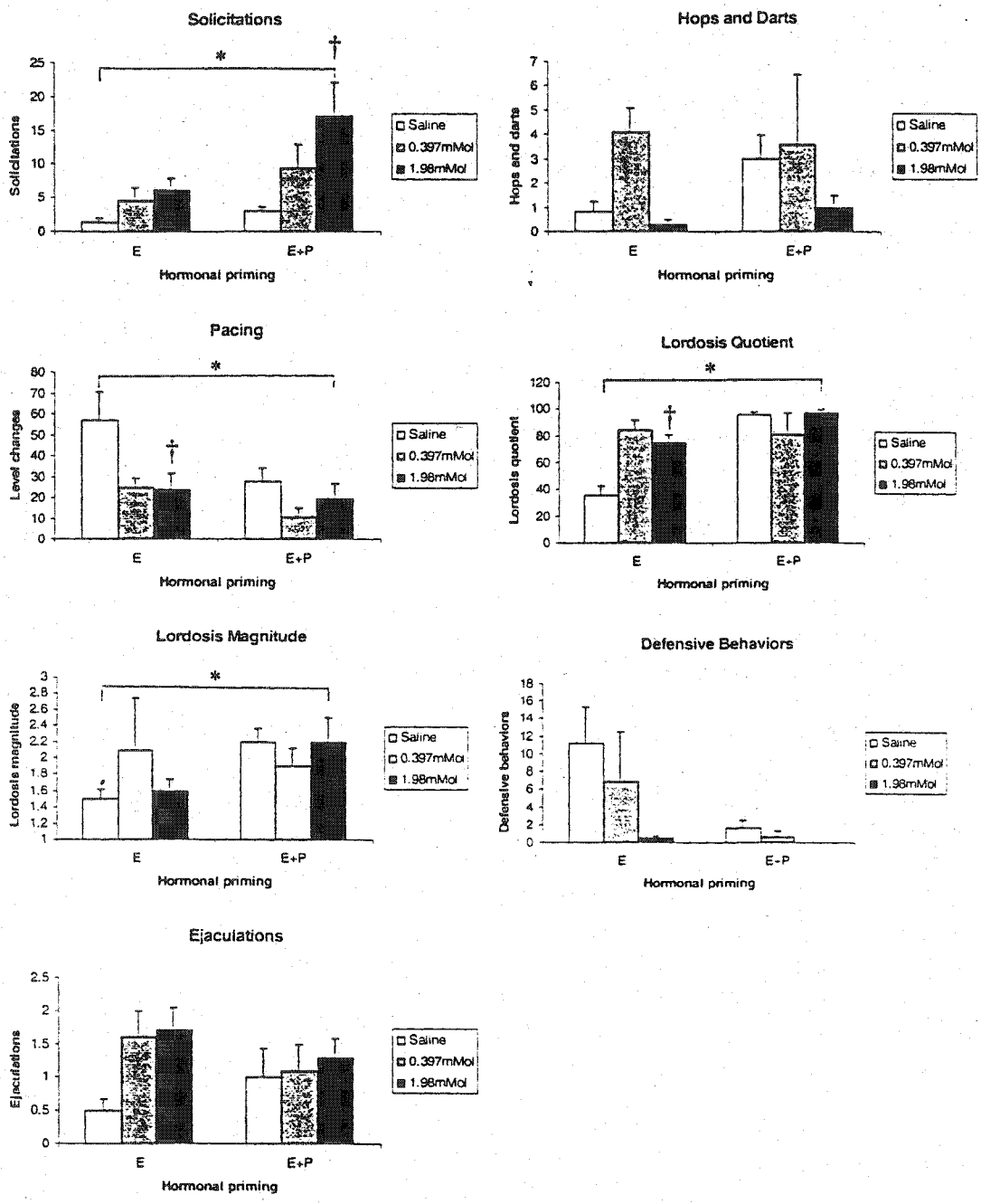

Figure 9. The effect of 2 doses of DNQX or saline infusions on the mean number of solicitations, hops and darts, level changes, mean lordosis magnitude, mean lordosis quotient, mean number of defensive behaviors and male ejaculations. Error bars represent standard errors. († = $p < .05$, effect of dose: saline vs 0.397mMol vs 1.98mMol, within subjects; * = $p < .05$, effect of hormonal priming: E vs E+P, between subjects)

dose was also detected, $F(2, 30) = 4.00$, $p < 0.05$; *post hoc* analyses revealed that animals that received the large dose of DNQX level changed significantly less than animals that received the saline infusion.

Lordosis quotient. The ANOVA detected a significant main effect of hormone, $F(1, 13) = 9.86$, $p < 0.01$, *post hoc* analyses revealing that animals in the E+P group obtained larger LQs than animals in the E-alone group. The ANOVA also detected a significant main effect of dose, $F(2, 26) = 4.79$, $p < 0.05$, *post hoc* analyses revealing that animals that were infused with DNQX obtained significantly larger LQs than animals that were infused with saline. Furthermore, the ANOVA detected a significant interaction between hormone and dose, $F(2, 26)$, $p < 0.001$. Post hoc analyses revealed that E-primed females who received saline infusions had significantly lower LQs than animals in all the other groups.

Lordosis magnitude. A significant main effect of hormone was detected by the ANOVA, $F(1, 12) = 6.01$, $p < 0.05$. *Post hoc* analyses revealed that animals primed with E+P obtained significantly higher LQs than animals primed with E-alone. A significant interaction between hormone and dose was also detected, $F(2, 24) = 3.64$, $p < 0.05$, however none of the mean differences reached the level of significance required by the Tukey. It appears as though infusions of the small dose of DNQX increased LMs relative to saline infusions in the E-alone group, whereas in the E+P group, the opposite effect is observed.

Defensive behaviors and hops and darts. Infusion of DNQX had no effect on these behaviors according to the ANOVAs.

Ejaculations. A significant main effect of dose was detected by the ANOVA, $F(2, 30) = 3.54$, $p < 0.05$, *post hoc* analyses revealing that males placed with females treated with the large dose of DNQX ejaculated more often than did males placed with females infused with saline.

EXPERIMENT 3

Results from Experiment 2, which investigated the behavioral effects of selective glutamate receptor antagonists, mirrored the results from Experiment 1 such that the antagonists facilitated sexual behaviors following infusion to the VMH of female rats primed with E. One hypothesis is that glutamate is normally active in females when they are not in heat, but inhibited by E-induced neurochemical changes. This inhibition may decline progressively during copulation, such that the reactivation of glutamate neurons may subserve part of the phenomenon of estrus termination. Because c-fos is induced in VMH glutamate neurons during VCS (Pfaus and Sabongui, 1996) and because a sodium channel blocker administered to the VMH attenuates the effects of VCS on lordosis (Dobbek and Pfaus, 2001), Experiment 3 investigated whether NMDA receptors in the VMH are involved in estrus termination induced by manual VCS.

Method

Subjects

Forty females and 20 males were obtained and housed in the same conditions as those in experiment 1 and 2. Sexual training trials, surgical and histological procedures were performed in the same manner as those in the first and second experiments.

Experimental procedure

The females were ovariectomized, hormonally primed with E+P and given 10 sexual trials with the sexually vigorous stimulus males. After the 10th trial of sexual behavior, females were taken off hormones for 26 days, receiving E at 13:00 on this 26th day, P at 17:00 on the 28th day, and VCS or sham VCS at 09:00 on this same 28th day. The highest dose of AP-5 from Experiment 2 was employed to assess the effect of a

selective glutamate receptor antagonist on estrus termination induced by VCS. VCS and sham VCS were given in the same manner as Pfaus et al. (1996; 2000) used to examine Fos induction and behavioral estrus termination. Briefly, VCS consisted of 50 manual VCSs with a lubricated glass rod in clusters of 5, each spaced 5 to 10 seconds apart. Clusters were distributed at 6-minute intervals over the course of one hour. Rats who received Sham VCS were held by the base of the tail, the tail and rump raised and anogenital region exposed for an amount of time equal to that necessary for the application of 5 VCSs, distributed over one hour.

The bilateral cannulations to the VMH were performed between the 10^{th} and the 19^{th} day of the 26 day of no hormone treatment period, ensuring that animals had enough time to recover from surgery before testing. Once cannulated, females were randomly assigned to one of 4 groups: group 1 was infused with a phosphate buffer just prior to sham VCS treatment; group 2 received the same phosphate buffer infusion followed by VCS. Group 3 received an AP-5 infusion followed by sham VCS, whereas group 4 received an AP-5 infusion followed by VCS. All rats received their estrus termination behavioral testing the following day at 09:00. In this experiment, the manner in which behavioral testing occurred differs from that followed in Experiments 1 and 2. Each female was placed in the bilevel chamber for 5 minutes prior to the introduction of the male. This male was allowed to copulate to one ejaculation, or 10 minutes, whichever came first, and was then removed from the chamber and replaced 5 minutes later by a second male who was allowed to copulate for 10 minutes, or until ejaculation, as was the case with the first male. These males were both sexually experienced.

This change in testing procedure led to changes in the behavioral analyses. Two sets of data were obtained from each female: one in presence of the first male and one in presence of the second male. The behavioral data of the female obtained during the five minutes of anticipation prior to each male's introduction to the bilevel chamber were included respectively in each data set. Once the behavioral data were collected, females were perfused, brains were removed, and the coronal slices mounted on gel-coated slides were analyzed under the microscope for cannulae placement verification, reported in Figure 10. Out of 40 females, 24 had correct bilateral placements, 14 had unilateral placements, and 2 animals died before testing occurred.

Statistical analyses

A 2 (stimulation: VCS vs. sham) x 2 (treatment: AP-5 vs. saline) x 2 (trial: male 1 vs. male 2) mixed factorial ANOVA with repeated measures on the third factor was conducted in order to assess the effects of AP-5 and VCS on the various aspects of female rat sexual behavior. Tukey *post hoc* analyses were conducted when statistical significance was detected to further indicate the location and direction of the significant mean differences. Only significant main effects and interactions between these variables are reported.

Results

Behavioral observations

As expected, females that received VCS prior to the behavioral testing were slightly more difficult to handle than females that received sham VCS. Otherwise, no unusual behaviors were observed.

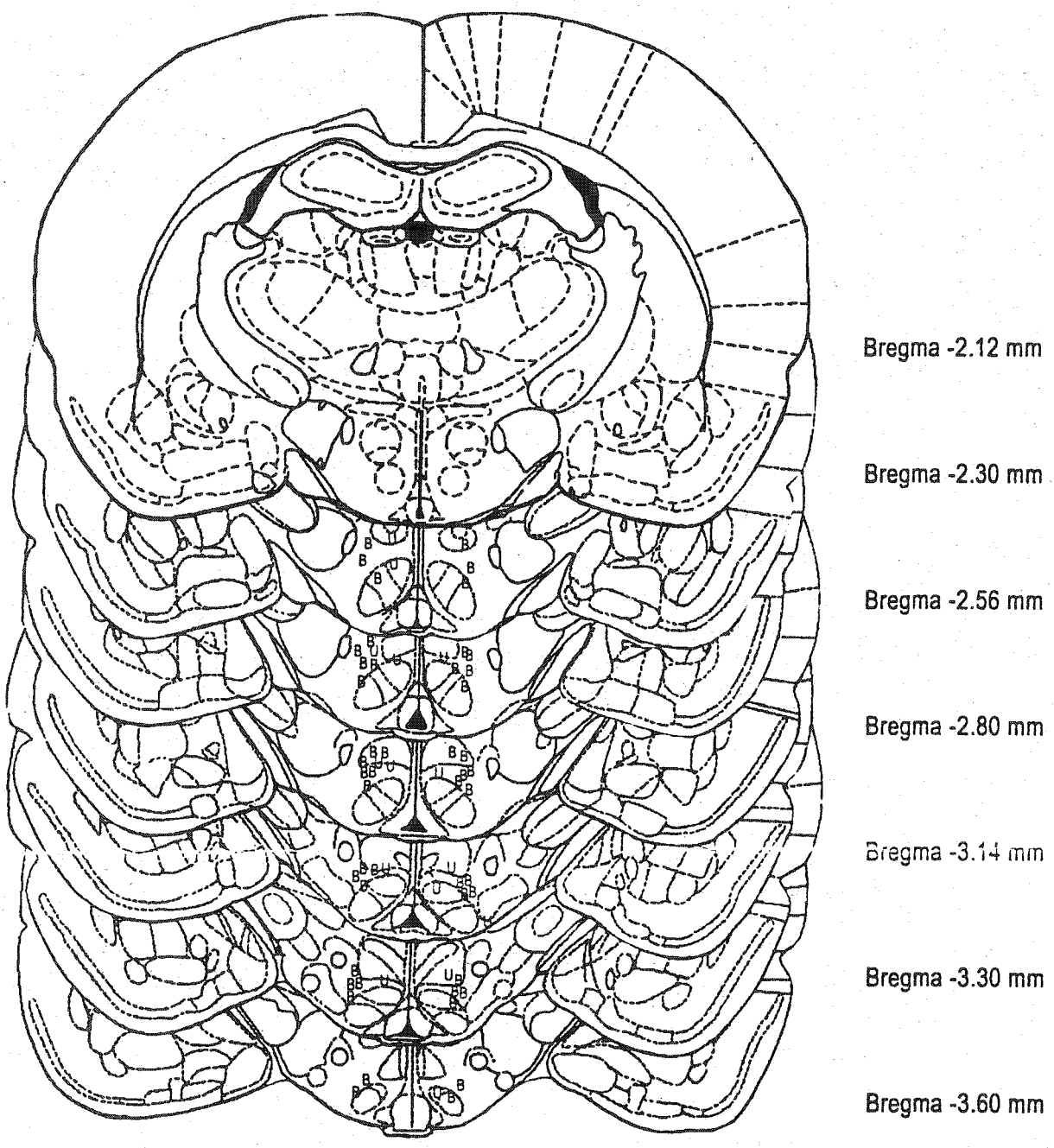

Figure 10. Placement data for subjects in Experiment 3. Correct bilateral cannulations are indicated by the letter "B," whereas correct unilateral cannulations are indicated By the letter "U."

The effect of VCS vs. sham VCS, AP-5 vs. saline infusions, and copulation with the first male introduced in the bilevel chamber vs. the second one on the number of solicitations, H&D, pacing, LQ, defensive behaviors and male ejaculations are shown in Figure 11. An ANOVA could not be conducted on LM due to absence of lordosis in a large number of females in the VCS group. Overall, when animals were infused with AP-5 and received VCS, they displayed fewer solicitations and H&D, but more defensive behaviors and lower LQs than rats infused with saline. Moreover, when females copulated with the first male, they solicited more, paced more and displayed less defensive behaviors than when they copulated with the second male.

Solicitations. A significant main effect of trial was detected, $F(1, 33) = 7.28$, $p < 0.05$, with females soliciting sex more from the first male than from the second. The 3-way interaction was also significant $F(1, 33) = 6.98$, $p < 0.05$. A Tukey *post hoc* analysis revealed that when females were infused with AP-5, the ones that received VCS solicited sex significantly less from the first male than did females who received sham VCS.

Hops and darts. The ANOVA did not detect any significant main effects of stimulation, treatment, or trial, nor did it detect any significant interactions between these three variables.

Pacing. A significant main effect of stimulation was detected, $F(1, 33) = 19.85$, $p < 0.001$, such that animals that received VCS changed levels less than animals that received sham VCS. The main effect of trial was also significant, $F(1, 33) = 5.57$, $p < 0.05$; *post hoc* analyses revealed that females level changed more often in presence of the first male than they did in presence of the second male. Furthermore, the ANOVA indicated that the interaction between stimulation and treatment was also significant, F(1,

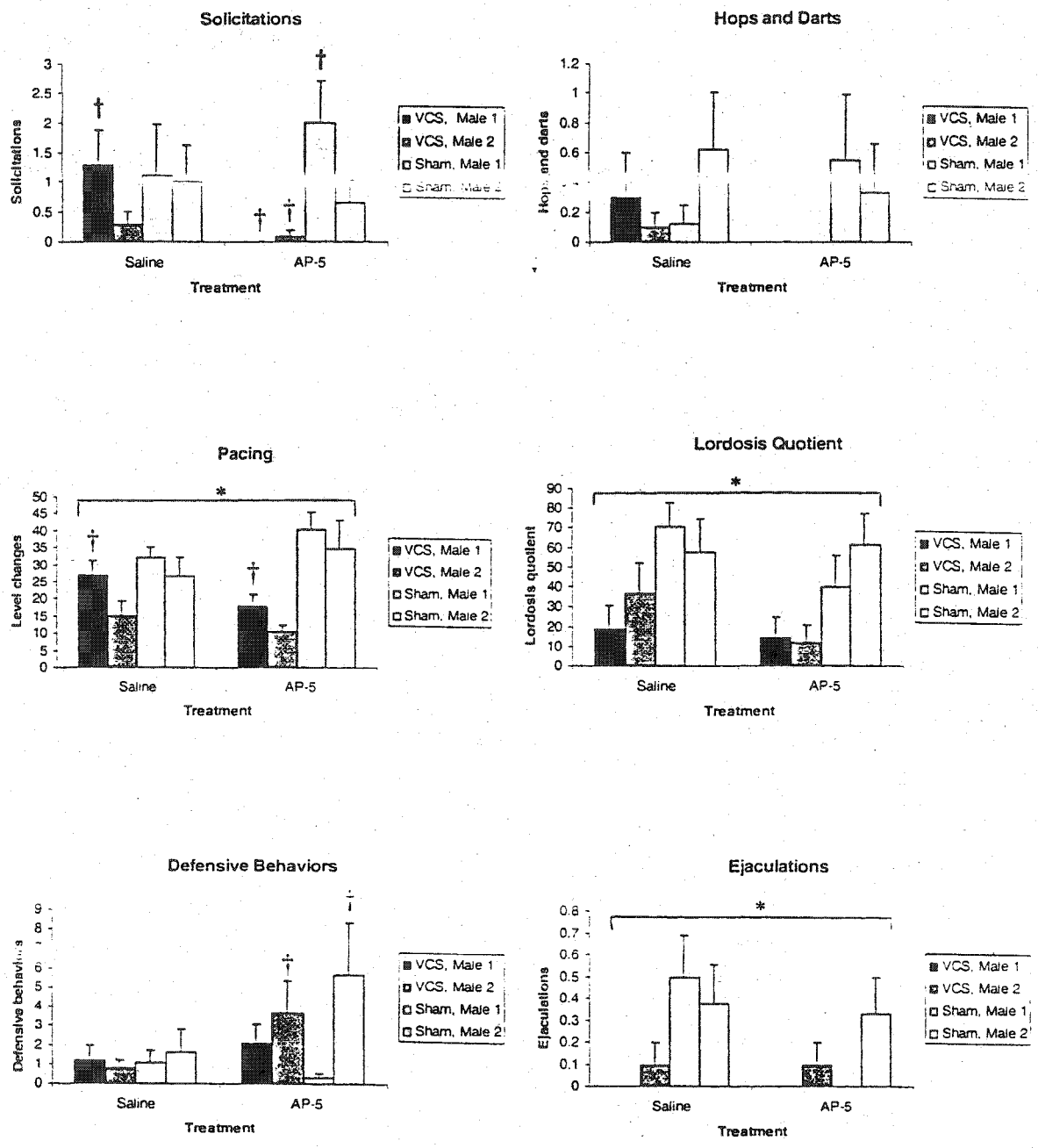

Figure 11. The effect of AP-5 or saline infusions on the mean number of solicitations, hops and darts, level changes, mean lordosis quotient, mean number of defensive behaviors and male ejaculations as a function of stimulation and trial. Error bars represent standard errors. (* = $p < .05$, effect of stimulation: VCS vs SHAM, between subjects; † = $p < .05$, effect of treatment: drug vs. saline, between subjects; ‡ = $p < .05$, effect of trial: male 1 vs male 2; within subjects)

33) = 4.35, $p < 0.05$. *Post hoc* analyses revealed that animals that received AP-5 infusions changed levels significantly less when they received VCS than when they received sham VCS, whereas in the groups that received saline infusions, the same pattern was not detected.

Lordosis quotient. A significant main effect of stimulation was found, $F(1,33) = 12.65$, $p < 0.01$. *Post hoc* analyses revealed that animals that received VCS had significantly lower LQs than those who received sham VCS. No other significant main effects or interactions were detected.

Defensive behaviors. The ANOVA detected a significant main effect of trial, $F(1, 33) = 4.69$, $p < 0.05$. *Post hoc* analyses revealed that animals displayed more defensive behaviors when placed with the second male than they did with the first. The ANOVA also indicated that the interaction between treatment and trial was significant, $F(1, 33) = 4.42$, $p < 0.05$. *Post hoc* analyses revealed that animals infused with AP-5 engaged in more defensive behaviors when placed with the second male than they did in presence of the first male. These analyses further indicated that females infused with AP-5 engaged in significantly more defensive behaviors while in presence of the second male than were rats infused with saline in presence of the first or second male.

Ejaculations. The ANOVA detected a significant main effect of manipulation on ejaculations, $F(1, 33) = 1.53$, $p < 0.01$. *Post hoc* analyses further revealed that when males were placed with females that received VCS, they ejaculated less frequently than they did when placed with females that received sham VCS. No other significant main effects or interactions were detected.

Discussion

Previous research suggested that the brain's main excitatory neurotransmitter glutamate, and one of its selective receptor agonists kainate, have an inhibitory effect on lordosis when administered bilaterally to the VMH of female rats (Kow et al., 1985). The purpose of the series of experiments reported in this thesis was to further investigate the role of glutamate receptors within the VMH by analyzing the behavioral effects that would ensue following administration of glutamate, its selective receptor agonists or antagonists to this region. Experiment 1 investigated the effect of glutamate and its three receptor agonists AMPA, NMDA and kainate on female sexual behavior. The results confirmed Kow and colleagues' suggestion 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.

Experiment 2 investigated the behavioral effects of three glutamate receptor antagonists, AP-5, CNQX and DNQX. The results indicate that selective glutamate receptor antagonists facilitate copulatory behavior when a low level of sexual functioning is induced by hormonal priming with E-alone, and to a lower extent when sexual functioning is already high due to hormonal priming with E+P. It appears that some of the antagonists' effects in E-primed females mimic the additive effects of P when administered in conjunction with E. Because selective glutamate receptor antagonists also facilitated sexual behavior in E+P-primed females, it is possible that they accentuate the efficacy with which P heightens sexual receptivity when administered in conjunction with E. Overall, the behavioral data pattern obtained following bilateral infusions of selective glutamate receptor antagonists to the VMH mirrored that of the agonists.

Experiment 3 was conducted to investigate whether the inhibitory mechanisms originating from glutamate receptors within the VMH are involved in estrus termination. The results suggest that they are not. Surprisingly, it seems as though AP-5, the NMDA selective antagonist, actually works to enhance some of the effects of VCS on female sexual behavior, contrary to the initial hypothesis. A more detailed discussion of the results of each experiment and their implication follows.

Contrary to the findings of Kow et al. (1985), bilateral microinfusions of glutamate to the VMH did not have an inhibitory effect on lordosis. In fact, frequencies of solicitation, pacing, defensive behaviors, lordosis quotients and magnitudes, and ejaculations by the males remained unaffected by administration of glutamate to the VMH; however, the number of hops and darts was significantly decreased. The differences in the results obtained by Kow et al. and those of the present study may be explained by the different methodologies used: Kow et al. primed OVX rats with silastic E capsules implanted subcutaneously. This hormone replacement regimen induces a lower level of sexual receptivity than full priming with E and P. Perhaps the low levels of lordosis induced by E 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 as opposed to the manual flank stimulation used by Kow et al. Perhaps female rats are more responsive to glutamate administered in the VMH when they receive manual flank stimulation relative to the olfactory, flank and vaginocervical stimulation they receive from a sexually vigorous male. 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 received in the Kow et al. study.

Infusions of kainate to the VMH had more pronounced effects on sexual behavior than glutamate. For instance, 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 substantially following kainate infusions, suggesting that females were trying to avoid sexual contact. Although kainic acid is highly excitotoxic to the brain, the largest dose administered was ten times smaller than that necessary for lesionning to occur (Stubley-Weatherly, Harding and Wright, 1996). One study indicated that the effect of infusions of a subtoxic dose (60pmol) of kainate in 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 the subsequent changes observed on other behaviors could have caused the reduction in sexual behavior that was detected in the present experiment.

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.

The purpose of the second experiment was to assess the behavioral effects of the NMDA or the AMPA/kainate receptor antagonists on females primed with E alone or E+P. The NMDA receptor antagonist AP-5 seems to increase the level of appetitive behaviors in both E- and E+P-primed females: the number of solicitations was significantly increased by the low dose of AP-5 in both hormonal priming conditions, whereas the number of hops and darts was significantly increased by infusion of the large dose in E+P primed females. These results suggest that AP-5 may further increase P's ability to induce proceptivity when administered in conjunction with E. The number of solicitations, lordosis quotients and lordosis magnitudes were all significantly larger when females were primed with E+P as compared to E, whereas the number of defensive behaviors was greater, following both saline and AP-5 infusions. These results are consistent with previous research showing that priming with E induces lower levels of sexual proceptivity and receptivity than E+P priming (Whalen, 1974, Pfaus et al., 1999). The interaction between hormone and dose on lordosis quotients which indicates that E+P-primed females obtained larger lordosis quotients than E-primed females when

infused with saline or the small dose of AP-5 provides further evidence for this finding. AP-5 did not produce a substantial effect on pacing, lordosis quotients, lordosis magnitudes, defensive behaviors or ejaculations, relative to saline baseline measurements in rats primed with E or E+P, suggesting that the NMDA receptor may be involved in the regulation of sexual proceptivity.

The AMPA/kainate receptor antagonist CNQX produced a progesterone-like effect on lordosis quotients in E-primed females: when infused with the large dose of CNQX, lordosis quotients almost reached the levels observed in E+P-primed animals. However, lordosis magnitudes were not increased, suggesting that CNQX affects the initiation of lordosis, but not the amplitude of the reflex. It is also interesting to note that the numbers of solicitations and hops and darts were increased by CNQX infusions, but not significantly, in both E- and E+P- primed females. The main effects of hormonal priming were significant for all examined behaviors except the number of ejaculations by males, such that E+P-primed females engaged in more solicitation and hoping and darting, obtained larger lordosis quotients and magnitudes, however paced less, and displayed less defensive behaviors that did animals primed with E-alone.

Bilateral infusions of DNQX to the VMH significantly increased the number of solicitations, decreased pacing, and increased the number of ejaculations by the males in both E- and E+P-primed females. Lordosis quotients were increased in E-alone primed females as well. These infusions did not have an effect on lordosis magnitudes or defensive behaviors, however a trend was detected towards increase in the number of hops and darts. Hormonal priming with E+P resulted in larger frequencies of solicitations, hops and darts, lordosis quotients and magnitudes, and lower levels of

pacing than did priming with E-alone. Furthermore, the significant interactions between hormonal priming and treatment on lordosis quotients indicate that DNQX infusions were able to increase lordosis quotients when females were primed with E-alone but not with E+P. These findings hint to an E+P-induced ceiling effect on the ability to lordose. In other words, when sexual functioning is not optimal (i.e., following priming with E-alone), DNQX increases the proportion of females that lordose when mounted by males, but not when sexual functioning is optimal (i.e, following E+P priming). These data suggest that the AMPA/kainate receptor is involved in the regulation of solicitation, pacing, ability to lordose and incite male ejaculations.

Overall, the pattern of data obtained in the first and second experiments corroborates the suggestion by Kow et al. (1985) that there are sexually inhibitory mechanisms originating from glutamate receptors within the VMH of female rats. CNQX increased hops and darts and lordosis quotients significantly, and there was a trend toward increases in solicitation as well. DNQX increased solicitations, decreased pacing, increased lordosis quotients and ejaculations by the males, and there was a trend toward increase in hops and darts. However, it is impossible to say which receptor is involved in the regulation of which behavior from the antagonists' data set, because DNQX and CNQX are both selective receptor antagonists of the AMPA/kainate receptors. Data from the first experiment indicate that the AMPA receptor agonist decreased solicitations, hops and darts, lordosis magnitudes and lordosis quotients, whereas the kainate receptor agonist decreased solicitations, pacing, lordosis quotients, and trends toward decreases in lordosis magnitude and increases in defensive behaviors were detected. It is possible that if the sample sizes were increased, these trends would reach significance and one could

then say that CNQX increased solicitations, DNQX increased hops and darts, and that kainate decreased lordosis magnitudes and increased the display of defensive behaviors. At this point, it is impossible to say whether it is the AMPA or the kainate receptor that mediate the ability of females to incite male ejaculations.

Future research should investigate the effects of more selective receptor antagonists, such as 2,3-dihydro-6-nitro-7-sulphamoyl-benzo(f)quinoxaline (NBQX), the subtype AMPA selective antagonist, or GYKI 52466, the noncompetitive subtype AMPA selective antagonist in order to isolate behaviors that AMPA receptors mediate relative to those mediated by kainate receptors. Overall, these results suggest that selective glutamate receptor antagonists, CNQX and DNQX in particular, administered to the VMH, enhance various aspects of the female rat sexual proceptivity and receptivity.

Experiment 3 investigated whether glutamate receptors might play a role in estrus termination. Relative to sham VCS, VCS resulted in significant decreases in the number of solicitations, level changing, LQs, and ejaculations, suggesting that VCS was effectively administered (eg., as in Pfaus et al., 2000). Also, copulation with the second male introduced in the bilevel chamber was less vigorous than that with the first male, as indicated by significant decreases in the number of solicitations and level changes, and increases in defensive behaviors. A significant interaction between stimulation and treatment indicates that there was less pacing in the AP-5/VCS group compared to the AP-5/sham VCS. It is interesting to note however, that the number of defensive behaviors was larger in the AP-5/VCS group than in the AP-5/sham VCS group. Therefore, females in the AP-5/VCS group used defensive behaviors more than level changing whereas females in the AP-5/sham VCS group did the opposite. Furthermore, the number of

solicitations was significantly smaller in the group of females that received VCS versus those that received Sham VCS following infusions of AP-5 and while copulating with the first male. Contrary to the initial hypothesis, AP-5 failed to prevent VCS from decreasing the rates of solicitation. Also, females engaged in more defensive behaviors when they were infused with AP-5 and copulated with the second male than in any other condition. This interaction between treatment and male is puzzling and difficult to explain. Could it be that AP-5 infusions just prior to VCS may work to amplify the effects of VCS? Further support for this hypothesis comes from the observation that rats in the VCS/AP-5 condition had consistently lower, although not statistically significant, rates of solicitations (0.00 *vs.* 1.30), hops and darts (0.00 *vs.* 0.30), level changes (18.00 *vs.* 27.20), and lower lordosis quotients (14.29 *vs.* 18.57) than rats in the VCS/saline condition. The opposite was true in the sham VCS group, except for lordosis quotients and the number of ejaculations. These results suggest that opposite to the initial hypotheses, antagonism of the NMDA receptor by AP-5 may actually work in synergy with pathways activated by VCS within the VMH to inhibit sexual behavior. This suggestion is reminiscent of that proposed by Caba, Komisaruk and Beyer (1998) to explain how AP-5 injected intrathecally increased the magnitude and duration of VCS-induced analgesia. The analgesic effect of AP-5 combined with VCS was greater than the effects of AP-5 and VCS separately, suggesting that they acted synergistically.

The results of the third experiment show that AP-5 failed to reverse the effects of VCS on sexual behavior, which is surprising considering that in the second experiment, AP-5 significantly increased solicitations, lordosis quotients, and hops and darts in E-alone primed females. Instead of a role in estrus termination, perhaps

glutamate receptors are involved in the termination of lordosis and initiation of pacing that follows intromissions or ejaculation (Pfaus et al., 1999), and even in signaling the activation of estrus termination that will occur hours later, but not in estrus termination *per se*. Perhaps the NMDA receptor antagonized by AP-5 is not involved in this inhibition; rather, AMPA/kainate receptors may be. Further research should assess the effects of CNQX, DNQX and NBQX on estrus termination. Also, it is possible that selective glutamate receptor antagonists could have more potent effects on the pattern of sexual behaviors observed during estrus termination if they are infused to the VMH just prior to behavioral testing. This hypothesis requires further investigation.

Taken together, the three experiments of this thesis provide confirming and additional evidence that glutamate receptors within the VMH participate in the regulation of female sexual behavior by inhibiting appetitive and consummatory behaviors and activating pacing and defensive behaviors. However, the potential interaction between glutamate and GABA, and their control by estrogen and/or progesterone, require further clarification. It is clear that glutamate and GABA have opposing roles in the VMH: glutamate inhibits certain aspects of female sexual behavior (Kow et al., 1985) whereas GABA facilitates lordosis (McCarthy et al., 1991). A mutually antagonistic role of these two neurotransmitter systems is not unprecedented: studies on neuroprotection have established that NMDA and AMPA receptor antagonists, like GABA-A receptor agonists, limit or prevent ischemia-induced damage in the hippocampus (Gagliardi, 2000).

Finally, it is possible that P facilitates proceptive and receptive sexual behavior in female rats, in part, by inhibiting glutamate neurons in the VMH. Selective glutamate

receptor antagonists had an effect on sexual behavior similar to that of P (following administration of E). Indeed, E increases the transcription of GAD (McCarthy, 1995), the enzyme that synthesizes GABA. If P stimulates GABA release in the VMH, and/or augments binding to GABA-A receptors (DeLorey and Olsen, 1994), and if GABA inhibits glutamate neurons or other inhibitory substrates activated by glutamate, then a facilitation of lordosis and other sexual behaviors would be expected. In turn, as GABA release or binding decreases, and as VMH glutamate neurons are activated by increasing amounts of VCS, (Pfaus and Sabongui, 1996), VMH glutamate neurons may be disinhibited. The increased glutamate release may then lead to increased pacing and defensive responses along with decreased frequencies of appetitive and consummatory sexual behaviors as the number of ejaculatory series increases (Pfaus et al., 1999). It would be interesting to determine whether glutamate neurons in the VMH possess GABA receptors. It would also be interesting to consider that there may be interactions between E, P, glutamate, and OXY or NE in the VMH. It is important to bear in mind that interactions between these neurotransmitters and hormones can also involve other areas of the brain, such as the mPOA, the VTA, the MCG, and the amygdala, as research established that these regions also play important roles in the regulation of female sexual behavior (see Pfaus and Heeb, 1997, for review). These hypotheses remain speculative; further research is required to shed light on the role of glutamate receptors and their potential activation by other neurotransmitters or hormones. Microdialysis studies that analyze glutamate release within the VMH throughout multiple ejaculatory series or estrus termination should further elucidate the role of glutamate within this region on female sexual behavior.

References

Alvarez, E.O., and Ruarte, M.B. (2001). Role of glutamate receptors in the nucleus accumbens on behavioral responses to novel conflictive and non-conflictive environments in the rat. *Behavioral Brain Research, 123*, 143-153.

Beach, F.A. (1976). Sexual attractivity, proceptivity and receptivity in female mammals. *Hormones and Behavior, 7*, 105-138.

Bell, K. and Kalivas, P.W. (1996). Context-specific cross-sensitization between systemic cocaine and intra-accumbens AMPA infusion in the rat. *Psychopharmacology, 127*, 377-383.

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

Card, J.P. and Moore, R.Y. (1984). The suprachiasmatic nucleus of the golden hamster: Immunohistochemical analysis of cell and fiber distribution. *Neuroscience, 13*, 415-431.

Carter, C.S. 1992. Oxytocin and sexual behavior. *Neuroscience and Biobehavioral Reviews, 16*, 131-144.

Chan, A., Dudley, C.A., and Moss, R.L. (1984). Hormonal and chemical modulation of ventromedial hypothalamic neurons responsive to vaginocervical stimulation. *Neuroendocrinology, 50*, 51-58.

Condon, T.P. Ronnekleiv, O.K. and Kelly, M.J. (1989). Estrogen modulation of the α-1-adrenergic response of hypothalamic neurons. *Neuroendocrinology, 50*, 51-58.

DeLorey, T.M. and Olsen, R.W. (1994). GABA and glycine. Basic *Neurochemistry*. In Siegel, Agranoff, Albers, and Molinoff Eds., Raven Press, 389-399.

Di Ciano, P. and Everitt, B.J. (2001). Dissociable effects of antagonism of NMDA and AMPA/KA receptors in the nucleus accumbens core and shell on cocaine-seeking behavior. *Neuropsychopharmacology, 25*, 341-360.

Dobbek, N. and Pfaus, J.G. (2001). The Role of the Ventromedial Hypothalamus in Estrus Termination Induced by Vaginocervical Stimulation, Unpublished manuscript, Concordia University.

Dorner, G., Docke, F., and Moustafa, S. (1968). Differential localization of a male and a female hypothalamic mating center. *Journal of Reproduction and Fertility, 17*, 583-586.

Dudley, C.A., Rajendren, G., and Moss, R.L. (1996). Signal processing in the vomeronasal system: Modulation of sexual behavior in the female rat. *Critical Review of Neurobiology, 10*, 265-290.

Dyer, R.G. and Cross, B.A. (1972). Antidromic identification of units in the preoptic and anterior hypothalamic areas projecting directly to the ventromedial and arcuate nuclei. *Brain Research, 43*, 254-258.

Erskine, M.S. (1989). Solicitation behavior in the estrous female rat: A review. *Hormones and Behavior, 23*, 473-502.

Erskine, M.S. (1993). Mating-induced increases in FOS protein in preoptic area and medial amygdala of cycling female rats. *Brain Research Bulletin, 32*, 447-451.

Etgen, A.M. (1990). Intrahypothalamic implants of noradrenergic antagonists disrupt lordosis behavior in female rats. *Physiology and Behavior, 48*, 31-36.

Etgen, A.M. and Karkanias, G.B. (1994). Estrogen regulation of noradrenergic signaling in the hypothalamus. *Psychoneuroendocrinology, 19*, 603-610.

Etgen, A.M., Ungar, S., and Pettiti, N. (1992). Estradiol and progesterone modulation of norepinephrine neurotransmission: Implications for female reproductive behavior. *Journal of Neuroendocrinology, 4*, 255-271.

Fahrbach, S.E., Morrell, J.I., and Pfaff, D.W. (1989). Studies of ventromedial hypothalamic afferents in the rat using three methods of HRP application. *Experimental Brain Research, 77*, 221-233.

Frankfurt, M., Fuchs, E., and Wuttke, W. (1984). Sex differences in gamma-aminobutyric acid and glutamate concentrations in discrete rat brain nuclei. *Neuroscience Letters, 50*, 245-250.

Frye, C.A., Murphy, R.E., and Platek, S.M. (2000). Anti-sense oligonucleotides, for progestin receptors in the VMH and glutamic acid decarboxylase in the VTA, attenuate progesterone-induced lordosis in hamsters and rats. *Behavior and Brain Research, 115*, 55-64.

Gagliardi, R.J. (2000). Neuroprotection, excitotoxicity and NMDA antagonists. *Archives of Neuropsychiatry, 58*, 583-588.

Goy, R.W. and Phoenix, C.H. (1963). Hypothalamic regulation of female sexual behaviour: Establishment of behavioral oestrus in spayed guinea-pigs following hypothalamic lesions. *Journal of Reproduction and Fertility, 5*, 23-40.

Hansen, S., Stanfield, E.J., and Everitt, B.J. (1980). The role of ventral bundle noradrenergic neurones in sensory components of sexual behaviour and coitus-induced pseudopregnancy. *Nature, 286*, 152-154.

Hardy, D.F., and DeBold, J.F. (1971). Effects of mounts without intromission upon the behavior of female rats during the onset of estrogen-induced heat. *Physiology and Behavior, 7*, 643-645.

Inenaga, K., Kannan, H., Yamachita, H. Tribollet, E. Raggenbass, M. and Dreifuss, J.J. (1991). Oxytocin excites neurons located in the centromedial nucleus of the guinea-pig hypothalamus. *Journal of Neuroendocrinology, 3*, 569-573.

Insel, T.R. (1992). Oxytocin--a neuropeptide for affiliation: Evidence from behavioral, receptor autoradiographic, and comparative studies. *Psychoneuroendocrinology, 17*, 3-35.

Johnson, A.E., Coirini, H., Ball, G.F. and McEwen, B.S. (1989). Anatomical localization of the effects of 17β-estradiol on oxytocin receptor binding in the ventromedial hypothalamic nucleus. *Endocrinology, 124*, 207-211.

Kendrick, K.M., Keverne, E.B., Hinton, M.R., and Goode, J.A. (1992). Oxytocin, amino acid and monoamine release in the region of the medial preoptic area and bed nucleus of the stria terminalis of the sheep during parturition and suckling. *Brain Research, 569*, 199-209.

Kim, Y.I., Dudley, C.A., and Moss, R.L. (1988). α2-adrenoceptro-mediated inhibition of hypothalamic norepinephrine release. *Journal of Neuroscience, 13*, 3448-3455.

Kimura, H. and Kuriyama, K. (1975). Distribtuion of gamma-aminobutyric acid (GABA) in the rat hypothalamus: Functional correlates of GABA with activities of appetite controlling mechanisms. *Journal of Neurochemistry, 24*, 903-907.

Kow, L.M., Harlan, R.E., Shivers, B.D., and Pfaff, D.W. (1985). 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 Research, 341*, 26-34.

Kow, L.M., Johnson, A.E., Ogawa, S., and Pfaff, D.W. (1991). Electrophysiological actions of oxytocin on hypothalamic neurons in vitro: Neuropharmacologic characterization and effects of ovarian steroids. *Neuroendocrinology, 54*, 526-535.

Kow, L.M., and Pfaff, D.W. (1987). Responses of ventromedial hypothalamic neurons in vitro to norepinephrine: Dependence on dose and receptor type. *Brain Research, 413*, 220-228.

Lavoie, M. (2000). Possible role of the ventromedial hypothalamus in estrus termination following vaginocervical stimulation of the rat. Masters of Science Thesis, Biology, Concordia University.

Luine, V.N., Wu, V., Hoffman, C.S., and Renner, K.J., 1999. GABAergic regulation of lordosis: Influence of gonadal hormones on turnover of GABA and interaction of GABA with 5-HT. *Neuroendocrinology, 69*, 438-445.

Malsbury, C.W., Kow, L.M., and Pfaff, D.W. (1977). Effects of medial hypothalamic lesions on the lordosis response and other behaviors in female golden hamsters. *Physiology and Behavior, 19*, 223-237.

Mathews, D. and Edwards, D.A. (1977). Involvement of the ventromedial and anterior hypothalamic nuclei in the hormonal induction of receptivity in the female rat. *Physiology and Behavior, 19*, 319-326.

McCarthy, M.M. (1995). Estrogen modulation of oxytocin and its relation to behavior. *Advances in Experimental Medicine and Biology, 395*, 235-245.

McCarthy, M.M., Kaufman, L.C., Brooks, P.J., Pfaff, D.W., Schwartz-Giblin S. (1995). Estrogen modulation of mRNA levels for the two forms of glutamic acid decarboxylase (GAD) in female rat brain. *Journal of Comparative Neurology, 360*, 685-697.

McCarthy, M.M., Malik, K.F., and Feder, H.H. (1990). Increased GABAergic transmission in medial hypothalamus facilitates lordosis but has the opposite effect in preoptic area. *Brain Research, 507*, 40-44.

McCarthy, M.M., Pfaff, D.W., and Schwartz-Giblin, S. (1991). Midbrain central gray GABAA receptor activation enhances, and blockade reduces, sexual behavior in the female rat. *Experimental Brain Research, 86*, 108-116.

McClintock, M.K. (1984). Estrous synchrony: Modulation of ovarian cycle length by female pheromones. *Physiology and Behavior, 32*, 701-705.

Meeker, R.B., Greenwood, R.S., and Hayward, J.N. (1994). Glutamate receptors in the rat hypothalamus and pituitary. *Endocrinology,134*, 621-629.

Moore, R.Y. and Bloom, F.E. (1979). Central catecholamine neuron systems: Anatomy and physiology of the norepinephrine and epinephrine systems. *Annual Review of Neuroscience, 2*, 113-168.

Park, W.K., Bari, A.A., Jey, A.R., Anderson, S.M., Spealman, R.D., Rowlett, J.K., and Pierce, R.C. (2002). Cocaine administered into the medial prefrontal cortex reinstates cocaine-seeking behavior by increasing AMPA receptor-mediated glutamate transmission in the nucleus accumbens. *The Journal of Neuroscience, 22*, 2916-2925.

Pfaff, D.W. (1980). *Estrogens and Brain Function: Neural Analysis of a Hormone-Controlled Mammalian Reproductive Behavior*. New York: Springer-Verlag.

Pfaff, D.W. (1999). *Drive: Neurobiological and Molecular Mechanisms of Sexual Motivation*. Cambridge: The MIT Press.

Pfaff, D.W. and Keiner, M. (1973). Atlas of estradiol-concentrating cells in the central nervous system of the female rat. *Journal of Comparative Neurology, 151*, 121-158.

Pfaff, D.W. and Sakuma, Y. (1979a). Deficit in the lordosis reflex of female rats caused by lesions in the ventromedial nucleus of the hypothalamus. *Journal of Physiology, 288*, 203-210.

Pfaff, D.W. and Sakuma, Y. (1979b). Facilitation of the lordosis reflex of female rats from the ventromedial nucleus of the hypothalamus. *Journal of Physiology, 288*, 189-202.

Pfaff, D.W., Schwartz-Giblin, S., McCarthy, M.M., and Kow, L.M. (1994). Cellular and molecular mechanisms of female reproductive behaviors. In E. Knobil, J. Neill (Eds.), *The physiology of reproduction* (2nd ed.). New York: Raven, 107-220.

Pfaus, J.G. and Heeb, M. (1997). Implications of immediate-early gene induction in the brain following sexual stimulation of female and male rodents. *Brain Research Bulletin, 44*, 397-407.

Pfaus, J.G., Jakob, A., Kleopoulos, S.P., Gibbs, R.B., and Pfaff, D.W. (1994). Sexual stimulation induces Fos immunoreactivity within GnRH neurons of the female rat preoptic area: Interaction with steroid hormones. *Neuroendocrinology, 60*, 283-290.

Pfaus, J.G., Kleopoulos, S.P., Mobbs, C.V., Gibbs, R.B., and Pfaff, D.W. (1993). Sexual stimulation activates c-fos within estrogen-concentrating regions of the female rat forebrain. *Brain Research, 624*, 253-267.

Pfaus, J.G. and Sabongui, C. (1996). Vaginocervical stimulation induces Fos protein within glutamate neurons of the ventrolateral VMH. *Society for Neuroscience Abstracts, 22*, 917.

Pfaus, J.G., Smith, W.J., Byrne, N., and Stephens, G. (2000). Appetitive and consummatory sexual behaviors of female rats in bilevel chambers. II. Patterns of estrus termination following vaginocervical stimulation. *Hormones and Behavior, 37*, 96-107.

Pfaus, J.G., Smith, W.J., and Coopersmith, C.B. (1999). Appetitive and consummatory sexual behaviors of female rats in bilevel chambers. I. A correlational and factor analysis and the effects of ovarian hormones. *Hormones and Behavior, 35*, 224-240.

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

Polston, E.K., Erskine, M.S. (1995). Patterns of induction of the immediate-early genes c-fos and egr-1 in the female rat brain following differential amounts of mating stimulation. *Neuroendocrinology, 62*, 370-384.

Puma, C., Monmaur, V., Sharif, A., and Monmaur, P. (1996). 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. *Experimental Brain Research, 109*, 384-392.

Roullet, P, Sargolini, F., Oliverio, A., and Mele, A. (2001). NMDA and AMPA antagonist infusions into the v entral striatum impair different steps of spatial information processing in a nonassociative task in mice. *The Journal of Neuroscience, 21*, 2143-2149.

Rowe, D.W., Erskine, M.S. (1993). C-Fos proto-oncogene activity induced by mating in the preoptic area, hypothalamus and amygdala in the female rat: Role of afferent input via the pelvic nerve. *Brain Research, 621*, 25-34.

Rubin, B.S. and Barfield, R.J. (1983a). Induction of estrous behavior in ovariectomized rats by sequential replacement of estrogen and progesterone to the ventromedial hypothalamus. *Neuroendocrinology, 37*, 218-224.

Rubin, B.S. and Barfield, R.J. (1983b). Progesterone in the ventromedial hypothalamus facilitates estrous behavior in ovariectomized, estrogen-primed rats. *Endocrinology, 113*, 797-804.

Sakuma, Y. and Pfaff, D.W. (1983). Modulation of the lordosis reflex of female rats by LHRH, its antiserum and analogs in the mesencephalic central gray. *Neuroendocrinology, 36*, 218-224.

Silveira, M.C. and Graeff, F.G. (1992). Defense reaction elicited by microinjection of kainic acid into the medial hypothalamus of the rat: Antagonism by a GABAA receptor agonist. *Behavior and Neural Biology, 57*, 226-232.

Stubley-Weatherly, L., Harding, J.W., and Wright, J.W. (1996). Effects of discrete kainic acid-induced hippocampal lesions on spatial and contextual learning and memory in rats. *Brain Research, 716*, 29-38.

Stumpf, W.E. (1970). Estrogen-neurons and estrogen-neuron systems in the periventricular brain. *American Journal of Anatomy, 129*, 207-217.

Tetel, M.J., Getzinger, M.J., and Blaustein, J.D. (1993). Fos expression in the rat brain following vaginal-cervical stimulation by mating and manual probing. *Journal of Neuroendocrinology, 5*, 397-404.

Tetel, M.J., Getzinger, M.J., and Blaustein, J.D. (1994). Estradiol and progesterone influence the response of ventromedial hypothalamic neurons to tactile stimuli associated with female reproduction. *Brain Research, 646*, 267-272.

Tetel, M.J., Celentano, D.C., and Blaustein, J.D. (1994). Intraneuronal convergence of tactile and hormonal stimuli associated with female reproduction in rats. *Journal of Neuroendocrinology, 6*, 211-216.

Vathy, I. and Etgen, A.M. (1989). Hormonal activation of female sexual behavior is accompanied by hypothalamic norepinephrine release. *Journal of Neuroendocrinology, 1*, 383-388.

Vincent, P.A., and Etgen, A.M. (1993). Steroid priming promotes oxytocin-induced norepinephrine release in the ventromedial hypothalamus of female rats. *Brain Research, 620*, 189-194.

Wersinger, S.R., Baum, M.J., and Erskine, M.S. (1993). Mating-induced FOS-like immunoreactivity in the rat forebrain: A sex comparison and a dimorphic effect of pelvic nerve transection. *Journal of Neuroendocrinology, 5*, 557-568.

Whalen, R.E. (1974). Estrogen-progesterone induction of mating in female rats. *Hormones and Behavior, 5*, 157-162.