Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 86 (2007) 377–385
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

MK-801 infusions to the ventral tegmental area and ventromedial hypothalamus produce opposite effects on lordosis of hormone-primed rats

Sandra M. Petralia [a], Joseph F. DeBold [e], Cheryl A. Frye [a,b,c,d,*]

[a] *Department of Psychology, The University at Albany-SUNY, Albany, NY, USA*
[b] *Biological Sciences, The University at Albany-SUNY, Albany, NY, USA*
[c] *Center for Life Science, The University at Albany-SUNY, Albany, NY, USA*
[d] *Center for Neuroscience Research, The University at Albany-SUNY, Albany, NY, USA*
[e] *Department of Psychology, Tufts University, Medford, MA, USA*

Received 30 March 2006; received in revised form 6 October 2006; accepted 8 January 2007
Available online 13 January 2007

Abstract

Progesterone initiates female sexual behavior of rodents (lordosis) through actions at intracellular progestin receptors in the ventromedial hypothalamus. Progesterone's metabolite, 5α-pregnan-3α-ol-20-one, mediates the intensity and duration of lordosis through its actions at $GABA_A$ receptors in the ventral tegmental area. Whether progestins can influence sexual behavior through actions that involve *N*-methyl-D-aspartate receptors (NMDARs) in the ventromedial hypothalamus and ventral tegmental area was investigated. The current study examines the effect of bilateral ventral tegmental area or ventromedial hypothalamus infusions of the non-competitive NMDAR antagonist (+)-MK-801 hydrogen maleate (MK-801; 0, 20, or 200 ng) on lordosis, motor activity, and NMDA R1 subtype (NMDAR1) immunoreactivity in estradiol benzoate (10 μg)+progesterone (50 μg)- and estradiol benzoate+vehicle primed rats. Compared to vehicle infusions, infusions of MK-801 to the ventral tegmental area facilitated lordosis of estradiol benzoate (10 μg)+progesterone (50 μg)- and estradiol benzoate+vehicle primed rats. Infusions of MK-801 to the ventromedial hypothalamus inhibited lordosis of estradiol benzoate (10 μg)+progesterone (50 μg)- and estradiol benzoate+vehicle primed rats, compared to vehicle. There was no effect of MK-801 infusions to the ventral tegmental area or the ventromedial hypothalamus on motor behavior. Immunocytochemistry for NMDAR1 revealed MK-801 (200 ng) infusions to the ventral tegmental area or ventromedial hypothalamus of estradiol benzoate (10 μg)+progesterone (50 μg)- or estradiol benzoate+vehicle primed rats significantly reduced the number of darkly stained NMDAR1-immunoreactive cells, compared to vehicle infusions. These data suggest NMDARs may be important in the mediation of hormonal actions in both the ventral tegmental area and the ventromedial hypothalamus for sexual receptivity of rodents, but in different ways.

Keywords: *N*-methyl-D-aspartate receptors; Ventromedial hypothalamus; Ventral tegmental area; Lordosis

1. Introduction

Progesterone has actions to facilitate sexual receptivity of rats via intracellular estradiol-induced progestin receptors in the ventromedial hypothalamus, an important area of the brain for endocrine processing, and independent of progestin receptors in the midbrain ventral tegmental area, a critical neural substrate for motivation and reward signaling (Laviolette and van der Kooy, 2001; Wise and Rompre, 1989). Lesions to the ventromedial hypothalamus or ventral tegmental area, respectively, abolish or decrease estradiol- and progesterone-facilitated lordosis of rats (Herndon, 1976; Malsbury et al., 1977; Mathews et al., 1983). Implants to the ventromedial hypothalamus of estradiol and progesterone are sufficient to induce receptivity and proceptivity of rats (Rubin and Barfield, 1983a,b). Progesterone implants to the ventral tegmental area can enhance lordosis of estradiol-primed rats with or without implants to the ventromedial hypothalamus (Frye and Gardiner, 1996a; Pleim et al., 1991).

* Corresponding author. Department of Psychology, The University at Albany-SUNY, Life Sciences Research Building 01058, 1400 Washington Avenue, Albany, NY 12222, USA. Tel.: +1 518 591 8839; fax: +1 518 591 8848.
E-mail address: cafrye@albany.edu (C.A. Frye).

0091-3057/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2007.01.005

Progesterone's actions in the ventromedial hypothalamus and the ventral tegmental area for facilitation of sexual receptivity depend upon different mechanisms. Although there are many progestin receptors in the ventromedial hypothalamus, few progestin receptors have been localized to the ventral tegmental area (Frye, 2001a,b; Frye and Vongher 1999a). In addition, reducing progestin receptor immunoreactivity with infusions of anti-sense oligonucleotides to the ventromedial hypothalamus has an inhibitory effect on lordosis of naturally-receptive or hormone-primed rats (Mani et al., 1994; Frye and Vongher, 1999a,b; Frye et al., 2000). Whereas infusions of anti-sense oligonucleotides to the ventral tegmental area has no effect on lordosis of naturally-receptive or hormone-primed rats (Frye and Vongher, 1999a,b; Frye et al., 2000). Application of progestins to the ventral tegmental area that are impermeable to neuronal membranes (Frye and Gardiner, 1996a), or have low affinity for progestin receptors (Frye and Vongher, 1999b), facilitate lordosis rapidly. These effects of progestins are so rapid that it is unlikely that there is adequate time for transcription and/or translation of proteins, which progestins' actions at intracellular progestin receptors would require (Pfaff and McEwen, 1983). Progesterone's actions in the ventral tegmental area for modulating lordosis may require metabolism to 5α-pregnan-3α-ol-20-one (3α,5α-THP) and subsequent actions at various non-progestin receptor substrates, such as γ-aminobutyric acid type ($GABA_A$)/benzodiazepine receptor complexes (GBRs; Frye, 2001a,b). Interfering with progesterone's metabolism to 3α,5α-THP, the most effective endogenous GBR agonist (Harrison et al., 1987; Majewska et al., 1986), in the ventral tegmental area attenuates female sexual behavior of rats (Frye and Vongher, 2001). Infusions to the ventral tegmental area of GBR agonists or blockers respectively enhance and inhibit progesterone-facilitated lordosis of rats (Frye, 2001a, c; Frye and Vongher, 1999a). Thus, progesterone facilitates the onset and duration of receptivity in rodents via "genomic" effects, through progestin receptor interactions with DNA and induction of gene transcription, in the ventromedial hypothalamus. However, in the ventral tegmental area progesterone's actions occur via "non-genomic" or non-classical membrane-mediated effects that involve metabolism to 3α,5α-THP, and its actions at neuronal membranes, that produce rapid changes in ion flux and/or second messenger activity.

Progesterone may also have actions in the ventral tegmental area to modulate sexual receptivity by interacting with *N*-methyl-D-aspartate receptors (NMDARs). First, ventral tegmental area GABAergic neurons have NMDARs on them (Steffensen et al., 1998). Second, progestins can directly and indirectly modulate NMDARs. For example, NMDA binding, measured by autoradiography, is decreased in the frontal cortex of ovx rats following administration of progesterone or estradiol benzoate+progesterone (Cyr et al., 2000; Wu et al., 1991). As well, progestins can rapidly alter NMDAR function by increasing or decreasing NMDA-activated calcium influx and blocking progesterone's metabolism to 3α,5α-THP attenuates this effect (Gibbs et al., 1999; Smith, 1991). Progestins also indirectly alter NMDAR function in the hypothalamus and preoptic area by modulating the release of glutamate (Carbone et al., 1995; Fleischmann et al., 1990). Third, progesterone's actions at NMDARs in some areas of the brain can lead to functional changes. Notably, NMDA agonists produce excitatory responses in cerebellar Purkinje cells and persistent inflammatory hyperalgesia and administration of progesterone attenuates these effects by reducing NMDAR activity (Ren et al., 2000; Smith, 1991). Finally, NMDARs have been localized to the ventral tegmental area (Paquet and Smith, 2000) and pharmacological manipulations of NMDARs in the ventral tegmental area elicit electrophysiological and behavioral changes in rats (Narayanan et al., 1996; Willick and Kokkinidis, 1995; Zhang et al., 1992). Together these findings suggest progestins are capable of mediating changes in NMDAR function that produce alterations in behavior and leave open the possibility that, in the ventral tegmental area, progestins may modulate NMDARs, directly or indirectly, to produce changes in lordosis of rodents. This has been demonstrated by blocking NMDAR activity with systemic administration of (+)-MK-801 hydrogen maleate (MK-801) or dextrorphan — selective non-competitive antagonists at NMDARs. This treatment attenuates lordosis and motor behavior of ovariectomized (ovx) hormone-primed rats (Fleischmann et al., 1991) and hamsters (DeBold et al., 2000). As well, infusions to the ventral tegmental area of MK-801 (200 ng) to estradiol and progesterone-primed hamsters facilitates sexual receptivity over that seen with vehicle infusions (DeBold et al., 2000).

The purpose of this study is to examine whether NMDARs are a substrate for estradiol and/or progesterone actions in the ventromedial hypothalamus or ventral tegmental area to modulate sexual receptivity of rats. We hypothesize that if NMDARs are important in the ventromedial hypothalamus or ventral tegmental area for the expression of hormone-facilitated lordosis of rats, then infusions of MK-801 should alter sexual receptivity of rats. It is particularly important to ascertain the mechanisms underlying progestins actions for mediating reinforcing behaviors, given the potential role of progesterone in mediating response to drugs of abuse (Frye, submitted for publication). Furthermore, given that neuronal adaptations in the ventral tegmental area have been proposed to account for the progressive transition from non-dependence to drug dependence, model systems such as this (Kippin and van der Kooy, 2003) are particularly valuable as they are free from potential confounds of direct drug-related effects and can be used to examine neural substrates for particularly salient motivated behaviors (Berridge and Robinson, 1998; Koob and Le Moal, 2001; Spanagel et al., 1993).

2. Materials and methods

2.1. Animals

Subjects were 114 sexually-mature, female Long–Evans rats, which were bred from stock from Taconic Farms (Germantown, NY) and raised in our animal facility. Gonadally-intact, sexually-experienced male Long–Evans rats were used as stimulus animals for sexual receptivity testing. Rats were individually housed in the University Laboratory Animal Care Facility in the Social Sciences Building and were maintained on a 12:12 h dark/light cycle. Purina rat chow and tap water were freely available in the rats' cages. All experimental procedures were approved by the

Institutional Animal Care and Use Committee at the University of Albany.

2.2. Surgical procedures

Surgical procedures were conducted while subjects were anesthetized with Rhompun (60 mg/kg; Bayer Corp., Shawnee Mission, KS) and Ketaset (80 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA). Rats were ovx and stereotaxically implanted in one of three conditions. Some rats had 2 guide cannulae, aimed bilaterally to the ventral tegmental area (ventral tegmental area: ventral tegmental area). Other rats had 2 guide cannulae aimed bilaterally to the ventromedial hypothalamus (ventromedial hypothalamus: ventromedial hypothalamus). A third group of rats had 4 guide cannulae, 2 aimed bilaterally to the ventral tegmental area and 2 aimed bilaterally to the ventromedial hypothalamus (ventral tegmental area: ventral tegmental area/ventromedial hypothalamus: ventromedial hypothalamus). The coordinates for each region (ventral tegmental area: AP −5.3, ML±.04, DV −7.0; ventromedial hypothalamus: AP −2.8, ML±0.3, DV −7.8) were obtained from the stereotaxic atlas for the rat brain (Paxinos and Watson, 1986), and modified according to past hit rates. Cannula guides were constructed from 23-gauge stainless steel needles with 30-gauge removable inserts and fixed in place with dental cement. Following stereotaxic surgery, all rats were monitored to determined whether they were neurologically intact and had gained weight since the implant procedure (Marshall and Teitlebaum, 1974). Only rats meeting these criteria were included in this study.

2.3. Steroid preparation

Crystalline estradiol benzoate (estradiol benzoate) and progesterone were obtained from Sigma Chemical Co. (St. Louis, MO) and dissolved in corn oil to concentrations of 10 μg/ 0.2 ml and 50 μg/ 0.2 ml, respectively, for subcutaneous administration.

2.4. MK-801 infusions

(+)-MK-801 hydrogen maleate (a.k.a. dizocilipine maleate) was obtained from Sigma Chemical Co. (St. Louis, MO) and dissolved in sterile saline to concentrations of 0, 20 or 200 ng/0.5 μl, respectively, for intracranial administration. On the morning of testing, the concentrations of the drug were made and then stored at 4 °C until used (approximately 30 min). Bilateral infusions were performed with 5-μl Hamilton syringes attached to PE-20 tubing connected to a 30-gauge needle in awake animals (Frye and Duncan, 1994, 1995). Infusate volume was 0.5 μl/ infusion over 75 s. To minimize displacement of the infusate, the infusion needle remained in place for 180 s following infusion.

2.5. Behavioral testing

In order to examine effects on locomotion, immediately following infusion, rats were placed in a dimly-lit 39×39×30 cm Digiscan Optical Animal Activity Monitor (Accuscan Instruments Inc., Columbus, OH). The number of beam breaks that occurred in a 5-min test was mechanically recorded.

To assess sexual receptivity, experimental female rats were placed in a Plexiglas chamber (50×25×30 cm) with a sexually-experienced male. The male was allowed to mount the female 10 times or for a total interaction time of 10 min, whichever occurred first. Receptivity of rats was quantified by rating dorsiflexion during lordosis (0–3; Hardy and DeBold, 1971, 1972, 1973). Lordosis quotient [(# of lordosis responses/# of mounts)×100] and lordosis ratings were calculated and used for statistical analysis (Frye et al., 1996a,b). Inter-rater reliability for these indices of female sexual behavior in our laboratory has a concordance rate of greater than 95%.

2.6. Procedure

2.6.1. Experiment 1: effects of MK-801 infusions to the ventral tegmental area or ventromedial hypothalamus of estradiol benzoate+progesterone-primed rats on sexual behavior

After a one-week recovery period, ovx rats were tested following 10 μg estradiol benzoate priming at hour 0 and systemic progesterone (50 μg) at hour 44. At hour 47.5, rats were given bilateral infusions of MK-801 (20 or 200 ng) or vehicle in a 0.5 μl volume to the ventral tegmental area or the ventromedial hypothalamus. Immediately following infusion, rats were tested for motor behavior and sexual receptivity. For this experiment, 36 rats (n=18 bilateral ventral tegmental area or bilateral ventromedial hypothalamus) were tested for three weeks, once at each MK-801 dosage (0, 20, or 200 ng) following estradiol benzoate and progesterone (50 μg).

2.6.2. Experiment 2: effects of MK-801 infusions to the ventral tegmental area or ventromedial hypothalamus of estradiol benzoate-primed rats on sexual behavior

After a one-week recovery period, ovx rats were tested following 10 μg estradiol benzoate priming at hour 0. At hour 47.5, rats were given bilateral infusions of MK-801 (20 or 200 ng) or vehicle in a 0.5 μl volume to the ventral tegmental area or ventromedial hypothalamus. Immediately following infusion, rats were tested for motor behavior and sexual receptivity. For this experiment, a between subject design was utilized, there were 65 rats (n=36 bilateral ventral tegmental area and n=29 bilateral ventromedial hypothalamus). Each rat received a single dosage of MK-801 (0, 20, or 200 ng) to the ventral tegmental area (0 ng, n=12; 20 ng, n=12; 200 ng, n=12) or ventromedial hypothalamus (0 ng, n=9; 20 ng, n=10; 200 ng, n=10).

2.6.3. Experiment 3: effects of MK-801 infusions to the ventral tegmental area or ventromedial hypothalamus of estradiol benzoate+progesterone- or estradiol benzoate-primed rats on immunoreactivity

Following a one-week recovery period, rats with 4 cannulae guides received 10 μg estradiol benzoate priming at hour 0 and systemic progesterone (0 or 50 μg) at hour 44. At hour 47.5, rats were given bilateral infusions of MK-801 (0 or 200 ng) in a 0.5 μl volume to the ventral tegmental area and ventromedial

hypothalamus (ventral tegmental area: ventral tegmental area/ventromedial hypothalamus: ventromedial hypothalamus). Rats were killed at what would have been the test time to examine effects of hormonal state and MK-801 (0 or 200 ng) infusions on immunocytochemistry for the NMDA receptor R1 subtype (NMDAR1). For Experiment 3, $n=12$ rats (all with 4 guide cannulae, 2 bilateral to the ventral tegmental area and 2 bilateral to the ventromedial hypothalamus) received estradiol benzoate and progesterone (0 or 50 μg) followed by MK-801 (0 or 200 ng) to all 4 cannulae. These rats were not behaviorally tested but were killed in the condition analogous to the rats behaviorally tested in Experiments 1 and 2.

2.7. Histology

After behavioral testing (Experiments 1 and 2), rats were deeply anesthetized with sodium thiopental (150 mg/kg or to effect), intracardially exsanguinated with 0.9% phosphate buffered saline (PBS), and then perfused with formalin (10%). Brains were post-fixed in 30% sucrose-PBS and sliced at 40 μm in a cryostat. Sections through the targeted sites were stained with cresyl violet and examined to ascertain infusion location.

For examination of effects of MK-801 infusions on immunocytochemistry for NMDAR1, at what would have been the test time for rats in Experiments 1 and 2, those from Experiment 3 were deeply anesthetized with sodium thiopental (150 mg/kg or to effect), injected intracardially with heparin (1000 units), and perfused with 4% paraformaldehyde. Brains were post-fixed in paraformaldehyde overnight at 4 °C and then transferred to 30% sucrose-PBS the next day. After the brains sank, they were sliced at 40 μm in a cryostat. Free floating sections were washed in PBS (pH=7.4), hydrogen peroxide, and then incubated in normal horse serum for 1 h to decrease non-specific binding. The monoclonal antibody for NMDAR1 (Chemicon, MAB363, 1:1000; Gazzaley et al., 1996), which recognizes an epitope between amino acids 660–811 of the NMDAR1, was diluted in 0.1% bovine serum albumin (BSA)/PBS with 2% normal horse serum. The NMDAR1 monoclonal antibody MAB363 was chosen to examine the effects of hormone-priming and MK-801 infusions on NMDAR1-immunoreactivity because [^{3}H]MK-801 has been shown to bind specifically and to a single site on human embryonic kidney 293 cells that are transiently expressing NMDAR1, but not control cells (Chazot et al., 1992). This observation suggests MK-801 binds to the NMDAR1 to exert its inhibitory effects on NMDAR, and thus, assessment of NMDAR1 immunoreactivity would provide an appraisal of the degree to which MK-801 infusions to the ventromedial hypothalamus or ventral tegmental area blocked NMDAR function. Sections were then incubated for 48 h at 4 °C in the primary antibody. After this incubation, the sections were rinsed in normal horse serum, incubated in a biotinylated secondary antibody for 1 h, washed in PBS, and incubated in an avidin–biotin solution for 30 min in order to utilize a peroxidase staining kit for diaminobenzidine (Vector Laboratories, Burlingame, CA). Sections were rinsed in distilled water, rinsed in PBS, mounted on gelatin-coated slides, and coverslipped when dry.

The number of darkly stained immunoreactive (IR) cells in the ventral tegmental area or ventromedial hypothalamus were counted with a light microscope at 400× magnification by 2 investigators (interrater reliability was >90%). Staining for NMDAR1-IR was localized on somal and dendritic membranes. Immunoreactive cells between the borders of the interpeduncular nucleus, the red nucleus, and the medial lemniscus, and between the anterior to posterior extent of −5.1 to −5.5 mm, were considered in the ventral tegmental area, and cells adjacent to the arcuate nucleus of the hypothalamus on either side of the third ventricle, but not further than the lateral hypothalamus, were considered within the ventromedial hypothalamus [within the anterior to posterior range of −2.6 to 3.0 mm]. A reticle was superimposed over the ventral tegmental area and ventromedial hypothalamus to ensure that the same size areas were counted for all sections.

2.8. Statistical analyses

2.8.1. Experiment 1

Histological analysis for Experiment 1 revealed 17 of the 18 subjects had infusions to the ventral tegmental area. The data from the rat with bilateral infusions outside of the ventral tegmental area was excluded. Of the 17 with correct ventral tegmental area infusions, 11 had bilateral infusions and 6 had unilateral infusions. There were no behavioral differences produced as a function of unilateral vs. bilateral ventral tegmental area infusions; hence, these groups were combined. Similarly, 17 of the 18 subjects had infusions to the ventromedial hypothalamus and the data from the rat with bilateral infusions outside of the ventromedial hypothalamus was excluded. Of the 17 rats with infusions targeted to the ventromedial hypothalamus, 7 were unilateral and 10 were bilateral. There were no behavioral differences produced as a function of unilateral vs. bilateral ventromedial hypothalamus infusions; hence, these groups were combined.

Overall, one-within (0, 20, or 200 ng MK-801), one-between (ventral tegmental area or ventromedial hypothalamus) analyses of variance (ANOVAs) were used to examine effects on lordosis quotients and lordosis ratings and the number of beam breaks in the horizontal crossing task. *Post-hoc* tests (planned pairwise comparisons) were utilized where appropriate to determine group differences.

2.8.2. Experiment 2

Histological analysis for Experiment 2 revealed that of the 36 rats that had infusions aimed to the ventral tegmental area, 34 received infusions to the ventral tegmental area. Of these 34, 22 had bilateral and 12 had unilateral infusions. Two rats had infusions to sites other than the ventral tegmental and were excluded from the analyses. Of the 29 infusions aimed to the ventromedial hypothalamus, 27 were to the ventromedial hypothalamus. Data from 2 rats that received infusions to sites other than the ventromedial hypothalamus sites were excluded. Of the 27 rats with infusions to the ventromedial hypothalamus, 5 were bilateral and 12 were unilateral. Overall, two-way (MK-801 infusion X CNS site) between subject analyses of variance (ANOVAs) were used to examine effects on lordosis quotients and lordosis ratings and the number of beam breaks in the horizontal crossing task.

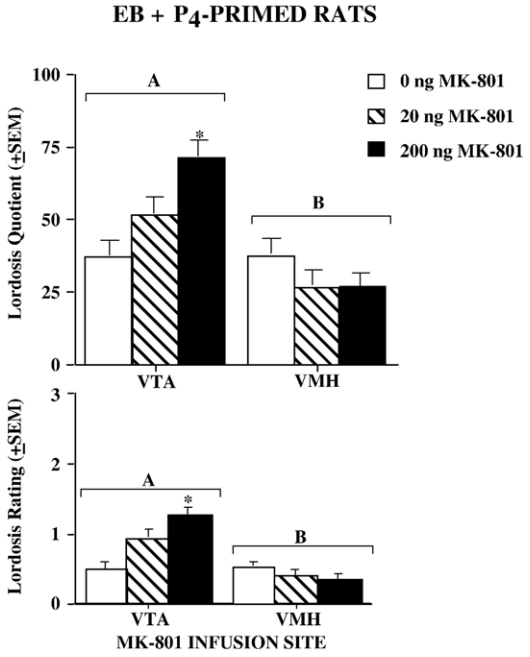

Fig. 1. Top panel represents mean lordosis quotients following ventral tegmental area (VTA; left) and ventromedial hypothalamus (VMH; right) infusions of 0 (open), 20 (striped), or 200 ng (black) MK-801 to estradiol benzoate (EB)+progesterone-primed rats. Bottom panel shows mean lordosis ratings following ventral tegmental area (left) and ventromedial hypothalamus (right) infusions of 0 (open), 20 (striped), or 200 ng (black) MK-801. Asterisks indicate a significant difference at $P<0.05$ between the indicated dosage of MK-801 and vehicle infusions. Different letters denote a significant difference at $P<0.05$ between infusion sites.

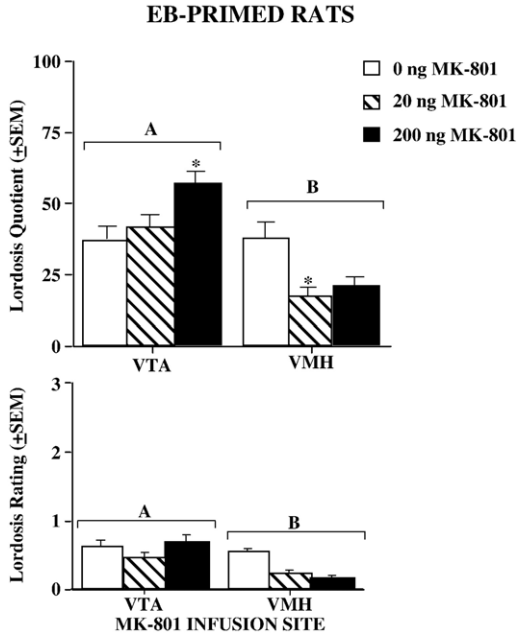

Fig. 2. Top panel represents mean lordosis quotients following ventral tegmental area (VTA; left) and ventromedial hypothalamus (VMH; right) infusions of 0 (open), 20 (striped), or 200 ng (black) MK-801 to estradiol benzoate (EB)-primed rats. Bottom panel shows mean lordosis ratings following ventral tegmental area (left) and ventromedial hypothalamus (right) infusions of 0 (open), 20 (striped), or 200 ng (black) MK-801. Asterisks indicate a significant difference at $P<0.05$ between the indicated dosage of MK-801 and vehicle infusions. Different letters denote a significant difference at $P<0.05$ between infusion sites.

Post-hoc tests (planned pairwise comparisons) were utilized where appropriate to determine group differences.

2.8.3. Experiment 3

The immunocytochemistry data were obtained to address the efficacy of MK-801 infusions and hormone-priming. Two-between (hormone regimen: estradiol benzoate or estradiol benzoate + P and infusion: MK-801 or vehicle), one within (CNS site: ventral tegmental area or ventromedial hypothalamus) analyses of variance (ANOVAs) were used to examine effects on NMDAR1-IR cells.

3. Results

3.1. Experiment 1: MK-801 infusions to the ventral tegmental area or ventromedial hypothalamus altered receptivity, but not motor behavior, of estradiol benzoate + progesterone-primed rats

There was a main effect for infusion site, ventromedial hypothalamus or ventral tegmental area, that showed infusions of MK-801 to the ventral tegmental area significantly increased lordosis quotients (55.0±6.6) [F (1,32)=13.66, $P<0.001$] and lordosis ratings (1.5±0.2) [F (1,32)=19.74, $P<0.0001$], compared to infusions to the ventromedial hypothalamus (lordosis quotients: 35.1±5.9; lordosis ratings: 0.5±0.1), of estradiol benzoate + progesterone-primed rats. There were no main effects of MK-801 dosage on lordosis quotients ($P=0.31$) or lordosis ratings ($P=0.58$). There was an interaction between infusion site and MK-801 dosage on lordosis quotients [F (2,64)=7.78, $P<0.001$] and lordosis ratings [F (2,64)=4.60, $P<0.01$]. *Post-hoc* analyses indicated these interactions are attributable to 200 ng infusions of MK-801 to the ventral tegmental area significantly increasing lordosis quotients and lordosis ratings over those seen with vehicle infusions to the ventral tegmental area or vehicle or MK-801 (20 and 200 ng) infusions to the ventromedial hypothalamus (see Fig. 1).

Table 1
Effect of MK-801 or vehicle infusions to the ventromedial hypothalamus or ventral tegmental area of estradiol benzoate and progesterone-primed rats on total number of beam breaks during a 5-min period in the horizontal crossing task

CNS site	MK-801 dosage			
	n	0 ng	20 ng	200 ng
Ventral tegmental area	17	1075.4±98.4	1176.4±127.7	977.4±72.6
Ventromedial hypothalamus	17	1061.4±87.4	1133.5±123.8	987.2±119.3

Table 2
Effect of MK-801 or vehicle infusions to the ventromedial hypothalamus or ventral tegmental area of estradiol benzoate-primed rats on total number of beam breaks during a 5-min period in the horizontal crossing task

CNS site	MK-801 dosage			
	n	0 ng	20 ng	200 ng
Ventral tegmental area	34	1050.1±98.4	1203.0±184.6	963.2±84.9
Ventromedial hypothalamus	27	876.38±215.2	1027.2±74.9	838.8±161.2

There was no effect of infusion site ($P=0.89$), MK-801 dosage ($P=0.14$), or any interaction between these variables ($P=0.95$) on the number of bream breaks in the horizontal crossing task (see Table 1).

3.2. Experiment 2: MK-801 infusions to the ventral tegmental area or ventromedial hypothalamus altered receptivity, but not motor behavior of estradiol benzoate-primed rats

There was a main effect for infusion site that showed infusions of MK-801 to the ventral tegmental area significantly increased lordosis quotients (44.1±8.5) [F (1,55)=8.72, $P<0.01$] and lordosis ratings (0.6±0.1) [F (1,55)=1.44, $P<0.01$] compared to infusions to the ventromedial hypothalamus (lordosis quotients: 24.6±6.7; lordosis ratings: 0.3±0.1) of estradiol benzoate-primed rats. There were no main effects of MK-801 dosage on lordosis quotients ($P=0.11$) and lordosis ratings ($P=0.25$), but there was an interaction between infusion site and MK-801 dosage on lordosis quotients [F (2,55)=3.55, $P<0.03$] but not lordosis ratings ($P<0.55$). *Post-hoc* analyses indicate this interaction is attributable to 200 ng infusions of MK-801 to the ventral tegmental area resulting in higher lordosis quotients than those seen with vehicle infusions to the ventral tegmental area and all ventromedial hypothalamus infusions, and to 20 ng infusions of MK-801 to the ventromedial hypothalamus producing lower lordosis quotients than those seen with vehicle infusions to the ventromedial hypothalamus and all ventral tegmental area infusions (see Fig. 2).

There was no effect of infusion site ($P=0.17$), MK-801 dosage ($P=0.30$), or an interaction ($P=0.97$) between these variables on the number of bream breaks in the horizontal crossing task (see Table 2).

3.3. Experiment 3: MK-801 (200 ng) infusions to the ventral tegmental area or ventromedial hypothalamus of estradiol benzoate+progesterone- or estradiol benzoate-primed rats decreased NMDAR1 immunoreactivity

Overall, immunocytochemistry for NMDAR1 revealed fewer NMDAR1-IR cells in the MK-801 infused rats [F (1,53)=51.253, $P<0.0001$] than in control rats. There were no differences in the number of NMDAR1-IR cells between estradiol benzoate and estradiol benzoate+progesterone-primed rats ($P=0.95$) or between ventral tegmental area and ventromedial hypothalamus ($P=0.31$) infusions (see Table 3).

Table 3
Effect of MK-801 or vehicle infusions to the ventromedial hypothalamus or ventral tegmental area of estradiol benzoate+progesterone- and estradiol benzoate-primed rats on the total number of darkly stained, NMDAR1-IR cells in the ventromedial hypothalamus or ventral tegmental area

	MK-801 dosage	Hormone condition	
		Estradiol benzoate+progesterone	Estradiol benzoate
Ventral tegmental area	0 ng	25.8±6.8	19.5±9.0
	200 ng	5.8±0.2 *	4.9±1.6 *
Ventromedial hypothalamus	0 ng	23.5±3.1	23.8±6.5
	200 ng	6.3±2.3 *	7.4±1.2 *

* Significant difference at $P<0.05$ between infusions of MK-801 (200 ng) and vehicle.

4. Discussion

These data support the hypothesis that NMDARs play an important role in regulating hormone-primed sexual receptivity of female rats, and that NMDARs in the ventral tegmental area and ventromedial hypothalamus play different roles in the modulation of lordosis of rats. In support, significantly higher lordosis quotients and lordosis ratings were observed in rats administered MK-801 infusions to the ventral tegmental area compared to MK-801 infusions to the ventromedial hypothalamus. Infusions of MK-801 (200 ng) to the ventral tegmental area increased lordosis quotients and lordosis ratings of estradiol benzoate+progesterone-primed rats, and lordosis quotients of estradiol benzoate-primed rats, above those seen with vehicle to the ventral tegmental area or vehicle or MK-801 to the ventromedial hypothalamus. Infusions of MK-801 to the ventromedial hypothalamus (20 ng) of estradiol benzoate-primed rats significantly decreased lordosis quotients below those seen with vehicle infusions to the ventromedial hypothalamus or infusions of vehicle or MK-801 to the ventral tegmental area. The effects of MK-801 infusions to the ventral tegmental area or ventromedial hypothalamus on sexual behavior of rats were not a consequence of disrupted motor behavior. MK-801 infusions did not significantly alter the total number of beam breaks in the horizontal crossing task. Finally, MK-801 infusions to the ventromedial hypothalamus or ventral tegmental area were effective at decreasing NMDAR1-IR cells compared to vehicle. Together, these data support the notion that NMDARs in the ventral tegmental area and ventromedial hypothalamus are important for the modulation of hormone-primed lordosis and that NMDA actions in these CNS sites may exert opposing influences to regulate sexual behavior of female rats.

These findings are consistent with previous work that shows pharmacological manipulations of hypothalamic NMDARs alter lordosis of rodents. In the present study, MK-801 infusions to the ventromedial hypothalamus produced a modest decrease in lordosis quotients of estradiol benzoate-primed rats but did not significantly decrease lordosis ratings of estradiol benzoate-primed rats or lordosis quotients and lordosis ratings of estradiol benzoate+progesterone-primed rats. Previous research shows decreasing excitatory amino acid activity in the mediobasal hypothalamus with infusions of D,L2-amino-5-phosphonopentoic acid (AP-5) did not alter lordosis of fully receptive female rats; however, more anterior infusions of AP-5 into the preoptic area of sexually receptive female rats did produce significant reductions in lordosis (McCarthy et al., 1991). Decreases in lordosis of rats have also been observed with intrahypothalamic infusions of non-specific glutamate agonists, NMDA, kainic acid, and potassium chloride (Kow et al., 1985). While the present findings, which utilize the glutamate antagonist specific to NMDARs, may seem inconsistent, one would not expect that the effects of exciting all glutamatergic neurons in the hypothalamus would be similar to a manipulation that expressly

blocks the NMDA subtype of glutamate receptors. Furthermore, findings that are similar to the current study, and previous work with rats, have been observed in hamsters. Indeed, decreases in total lordosis durations have been observed in ovx, estradiol benzoate+progesterone-primed hamsters following bilateral infusions of MK-801 (200 ng) to the ventromedial hypothalamus (DeBold et al., 2000). In sum, the current findings support previous research that indicates blockade of NMDA receptors in the hypothalamus has a modest inhibitory effect on lordosis of rodents. However, this study also extends present knowledge by examining the effects of NMDA antagonists in an area outside of the hypothalamus, the ventral tegmental area, on the lordosis of rats.

The present data suggest that NMDARs in the ventral tegmental area, in addition to those in the ventromedial hypothalamus, may be important substrates in the regulation of sexual behavior of female rats. In the current study, infusions of MK-801 (200 ng) to the ventral tegmental area produced increases in lordosis quotients and lordosis ratings of estradiol benzoate+progesterone- and lordosis quotients of estradiol benzoate-primed rats above those seen with vehicle infusions. Similar findings have been observed in ovx, estradiol benzoate (10 μg)+progesterone (50 μg)-primed hamsters, in which, infusions of MK-801 (200 ng) to the ventral tegmental area resulted in increases in total lordosis durations. Notably, blocking NMDARs in the ventral tegmental area produced increases in lordosis that are similar to those elicited by infusions of the GABA agonist muscimol into the ventral tegmental area of rats (Frye, 2001a,b) and hamsters (Frye and DeBold, 1992). NMDARs and GBRs in the ventral tegmental area may be substrates that modulate female sexual behavior perhaps, in part, through actions at dopaminergic neurons. Notably, in the ventral tegmental area, GBRs are present primarily on non-dopamine but also on some dopamine containing cell bodies (Churchill et al., 1992; Laviolette and van der Kooy, 2001, 2004; Rodriguez-Pallares et al., 2001) and NMDARs have been localized to soma, dendrites, and, to a lesser extent, small axons in the ventral tegmental area (Rodriguez et al., 2000). Manipulations of GBRs or NMDARs in the ventral tegmental area alter dopamine levels, which may lead to changes in lordosis. For example, ventral tegmental area infusions of the $GABA_A$ agonist, muscimol enhance lordosis (Frye, 2001a,b; Frye and DeBold, 1992) and decrease DA release in the prefrontal cortex and nucleus accumbens (Westerink et al., 1996, 1998). Similarly, ventral tegmental area infusions of MK-801 facilitated lordosis and reduced extracellular dopamine in the prefrontal cortex to levels that are 85% of controls (Westerink et al., 1998). It may be that, in the ventral tegmental area, NMDA neurons excite and GABAergic neurons inhibit dopaminergic cell bodies and that these opposing influences regulate dopamine concentrations in projection sites, which can have a modulatory effect on lordosis.

In summary, intracranial MK-801 had different effects on sexual receptivity depending on where it was infused. MK-801 had an inhibitory effect on sexual behavior in the ventromedial hypothalamus and a facilitatory effect on lordosis in the ventral tegmental area. This suggests NMDARs may have different roles in these two brain regions that are important in the hormonal control of rat receptivity. Estradiol- and/or progesterone-primed rats infused with MK-801 to the ventral tegmental area had increased lordosis, compared to vehicle infused rats, or rats that received MK-801 infusions to the ventromedial hypothalamus, which in fact decreased lordosis. These effects on lordosis were not secondary to non-specific changes in motor behavior, as there was no effect of MK-801 infusions to the ventral tegmental area or to the ventromedial hypothalamus on the number of beam breaks in the horizontal crossing task. Infusions of MK-801 to the ventromedial hypothalamus or ventral tegmental area effectively reduced NMDAR availability as shown by significant decreases in the number of NMDAR1-IR cells, compared to controls. Thus, NMDARs may be important substrates for mediating estradiol and/or progestin actions in the ventral tegmental area for effects on sexual receptivity of rats: similar mechanisms may underlie effects of these steroid hormones relevant for drugs of abuse.

Acknowledgments

This research was supported by the National Science Foundation (IBN 95–14463, 98–96263, 03–16083) and National Institutes of Mental Health (MH-067698). We appreciate the assistance provided by Joanna Wawrzyski for behavioral testing and Dr. Madeline Rhodes for immunocytochemistry.

References

Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Brain Res Rev 1998;28:309–69.

Carbone S, Szwarcfarb B, Losada M, Moguilevsky JA. Effect of ovarian hormones on the hypothalamic excitatory amino acids system during sexual maturation in female rats. Neuroendocrinol 1995;61:235–42.

Chazot PL, Miroslav C, Stephenson FA. Immunological detection of the NMDAR1 glutamate receptor subunit expressed in human embryonic kidney 293 cells and in rat brain. J Neurochem 1992;59:1176–8.

Churchill L, Dilts RP, Kalivas PW. Autoradiographic localization of gamma-aminobutyric acid A receptors within the ventral tegmental area. Neurochem Res 1992;17:101–6.

Cyr M, Ghribi O, DiPaolo T. Regional and selective effects of oestradiol and progesterone on NMDA and AMPA receptors in the rat brain. J Neuroendocrinol 2000;12:445–52.

DeBold JF, Parow AM, Fish EW. MK-801 infused into the ventral tegmental area of female hamsters enhances progesterone effects on sexual receptivity but inhibits in the ventromedial hypothalamus. SFN Abs 2000;26:1277.

Fleischmann A, Makman MH, Etgen AM. Ovarian steroids increase veratridine-induced release of amino acid neurotransmitters in preoptic area synaptosomes. Brain Res 1990;507:161–3.

Fleischmann A, Vincent PA, Etgen AM. Effects of non-competitive NMDA receptor antagonists on reproductive and motor behaviors in female rats. Brain Res 1991;568:138–46.

Frye CA. The role of neurosteroids and non-genomic effects of progesterone and androgens in mediating sexual receptivity of rodents. Brain Res Brain Res Rev 2001a;37:201–22.

Frye CA. The role of neurosteroids and non-genomic effects on progesterone-induced sexual behavior in rats and hamsters. Horm Behav 2001b;40:226–33.

Frye CA. Inhibition of 5α-reductase enzyme or $GABA_A$ receptors in the ventromedial hypothalamus and the ventral tegmental area attenuates

progesterone-induced sexual behavior in rats and hamsters. J Endocrinol Invest 2001c;24:399–407.

Frye CA. submitted for publication. Progestins influence motivation, reward, conditioning, stress, and/or response to drugs of abuse. Pharmacol Biochem Behav (special issue 2006).

Frye CA, DeBold JF. Muscimol facilitates sexual receptivity in hamsters when infused into the ventral tegmentum. Pharmacol Biochem Behav 1992;42:879–87.

Frye CA, Duncan JE. Progesterone metabolites, effective at the $GABA_A$ receptor complex, attenuate pain sensitivity in rats. Brain Res 1994;643:194–203.

Frye CA, Duncan JE. Estradiol benzoate potentiates neuroactive steroids' effects on pain sensitivity. Pharmacol Biochem Behav 1995;53:27–32.

Frye CA, Gardiner SG. Progestins can have a membrane-mediated action in rat midbrain for facilitation of sexual receptivity. Horm Behav 1996;30:682–91.

Frye CA, Vongher JM. $GABA_A$, D_1, and D_5, but not progestin receptor, antagonist and anti-sense oligonucleotide infusions to the ventral tegmental area of cycling rats and hamsters attenuate lordosis. Behav Brain Res 1999a;103:23–34.

Frye CA, Vongher JM. Progestins' rapid facilitation of lordosis when applied to the ventral tegmentum corresponds to efficacy at enhancing $GABA_A$ receptor activity. J Neuroendocrinology 1999b;11:829–38.

Frye CA, Vongher JM. Ventral tegmental area infusions of inhibitors of the biosynthesis and metabolism of 3α,5α-THP attenuate lordosis of hormone-primed and receptive rats and hamsters. J Neuroendocrinol 2001;13:1076–86.

Frye CA, van Keuren KR, Erskine MS. Behavioral effects of 3α-Androstanediol. I: Modulation of sexual receptivity and promotion of GABA-stimulated chloride flux. Behav Brain Res 1996a;79:109–18.

Frye CA, van Keuren KR, Rao PN, Erskine MS. Progesterone and 3α-androstanediol conjugated to bovine serum albumin affects estrous behavior when applied to the MBH and POA. Behav Neurosci 1996b;110:603–12.

Frye CA, Murphy RE, Platek SM. Anti-sense oligonucleotides, for progestin receptors in the ventromedial hypothalamus and glutamic acid decarboxylase in the ventral tegmental area, attenuate progesterone-induced lordosis in hamsters and rats. Behav Brain Res 2000;115:55–64.

Gazzaley AH, Weiland N, McEwen BS, Mottidon J,H. Differential regulation of NMDAR1 mRNA and protein by estradiol in the rat hippocampus. J Neurosci 1996;16:6830–8.

Gibbs TT, Yaghoubi N, Weaver CE, Park-Chung M, Russek SJ, Farb DH. Modulation of ionotropic glutamate receptors by neuroactive steroids. In: Baulieu EE, Robel P, Schumacher M, editors. Contemporary Endocrinology: Neuosteroids: A New Regulating Function In the Nervous System. Totowa NJ: Humana Press; 1999. p. 167–90.

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

Hardy DF, DeBold JF. Effects of coital stimulation upon behavior of the female rat. J Comp Physiol Psychol 1972;78:400–8.

Hardy DF, DeBold JF. Effects of repeated testing on sexual behavior of the female rat. J Comp Physiol Psychol 1973;85:195–202.

Harrison NL, Majewska MD, Harrington JW, Barker JL. Structure-activity relationships for steroid interaction with the gamma-aminobutyric acid A receptor complex. J Pharmacol Exp Ther 1987;241:346–53.

Herndon Jr JG. Effects of midbrain lesions on female sexual behavior in the rat. Physiol Behav 1976;17:143–8.

Kippin TE, van der Kooy D. Excitotoxic lesions of the tegmental pedunculopontine nucleus impair copulation in naive male rats and block the rewarding effects of copulation in experienced male rats. Eur J Neurosci 2003;18:2581–91.

Koob GF, Le Moal M. Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 2001;24:97–129.

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

Laviolette SR, van der Kooy D. $GABA_A$ receptors in the ventral tegmental area control bidirectional reward signaling between dopaminergic and non-dopaminergic neural motivational systems. Eur J Neurosci 2001;13:1009–15.

Laviolette SR, van der Kooy D. $GABA_A$ receptors signal bidirectional reward transmission from the ventral tegmental area to the tegmental pedunculopontine nucleus as a function of opiate state. Eur J Neurosci 2004;20:2179–87.

Majewska MD, Harrison NL, Schwartz RD, Barker JL, Paul SM. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 1986;232:1004–7.

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

Mani SK, Blaustein JD, Allen JMC, Law SW, O'Malley BW, Clark JH. Inhibition of rat sexual behavior by anti-sense oligonucleotides to the progesterone receptor. Endocrinology 1994;135:1409–14.

Marshall JF, Teitlebaum P. Further analysis of sensory inattention following lateral hypothalamic damage in rats. J Comp Physiolog Psychol 1974;86:375–95.

Mathews D, Donovan KM, Hollingsworth EM, Hutson VB, Overstreet CT. Permanent deficits in lordosis behavior in female rats with lesions of the ventromedial nucleus of the hypothalamus. Exp Neurol 1983;79:714–9.

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

Narayanan S, Willins D, Dalia A, Wallace L, Uretsky N. Role of dopaminergic mechanisms in the stimulatory effects of MK-801 injected into the ventral tegmental area and the nucleus accumbens. Pharmacol Biochem Behav 1996;54:565–73.

Paquet M, Smith Y. Presynaptic NMDA receptor subunit immunoreactivity in GABAergic terminals in rat brain. J Comp Neurol 2000;423:330–47.

Paxinos G, Watson C. The Rat Brain. New York: Academic Press; 1986.

Pfaff DW, McEwen BS. Actions of estrogens and progestins on nerve cells. Science 1983;219:808–14.

Pleim ET, Baumann J, Barfield RJ. A contributory role for midbrain progesterone in the facilitation of female sexual behavior in rats. Horm Behav 1991;25:19–28.

Ren K, Wei1 F, Dubner R, Murphy A, Hoffman GE. Progesterone attenuates persistent inflammatory hyperalgesia in female rats: involvement of spinal NMDA receptor mechanisms. Brain Res 2000;865:272–7.

Rodriguez JJ, Doherty MD, Pickel VM. *N*-methyl-D-aspartate (NMDA) receptors in the ventral tegmental area: subcellular distribution and colocalization with 5-hydroxytryptamine (2A) receptors. J Neurosci Res 2000;60:202–11.

Rodriguez-Pallares J, Caruncho HJ, Lopez-Real A, Wojcik S, Guerra MJ, Labandeira-Garcia JL. Rat brain cholinergic, dopaminergic, noradrenergic and serotonergic neurons express $GABA_A$ receptors derived from the α3 subunit. Receptors Channels 2001;7:471–8.

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

Rubin BS, Barfield RJ. Progesterone in the ventromedial hypothalamusfacilitates estrous behavior in ovariectomized, estrogen-primed rats. Endocrinol 1983b;113:797–804.

Smith SS. The effect of estrogen and progesterone on GABA and glutamate responses at extrahypothalamic sites. In: Costa E, Paul AM, editors. FIDA Research Foundation Symposium Series on Neurosteroids and Brain Function, vol. 8. New York: Theime Medical Publishers; 1991. p. 87–94.

Spanagel R, Almeida OF, Shippenberg TS. Long lasting changes in morphine-induced mesolimbic dopamine release after chronic morphine exposure. Synapse 1993;14:243–5.

Steffensen SC, Svingos AL, Pickel VM, Henriksen SJ. Electrophysiological characterization of GABAergic neurons in the ventral tegmental area. J Neurosci 1998;18:8003–15.

Westerink BH, Enrico P, Feimann J, De Vries JB. The pharmacology of mesocortical dopamine neurons: a dual-probe microdialysis study in the ventral tegmental area and prefrontal cortex of the rat brain. J Pharmacol Exp Ther 1998;285:143–54.

Westerink BH, Kwint HF, De Vries JB. The pharmacology of mesolimbic dopamine neurons: a dual-probe microdialysis study in the ventral tegmental area and nucleus accumbens of the rat brain. J Neurosci 1996;16:2605–11.

Willick ML, Kokkinidis L. The effects of ventral tegmental administration of $GABA_A$, $GABA_B$ and NMDA receptor agonists on medial forebrain bundle self-stimulation. Behav Brain Res 1995;70:31–6.

Wise RA, Rompre PP. Brain dopamine and reward. Annu Rev Psychol 1989;40:191–225.

Wu FS, Gibbs TT, Farb DH. Pregnenolone sulfate: a positive allosteric modulator at the *N*-methyl-D-aspartate receptor. Mol Pharmacol 1991;40:333–6.

Zhang J, Chiodo LA, Freeman AS. Electrophysiological effects of MK-801 on rat nigrostriatal and mesoaccumbal dopaminergic neurons. Brain Res 1992;590:153–63.