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Effects of manipulating progesterone and NMDA receptors in the ventral tegmental area for lordosis of hamsters and rats

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Abstract

Rationale—Progesterone (P_4) has actions in the ventral tegmental area (VTA) to regulate female sexual behavior in rodents. However, there are few intracellular progestin receptors (PRs) that have been identified in the VTA through which P_4 may have its actions to facilitate lordosis. There are *N*-methyl-D-aspartate receptors (NMDARs) in the VTA that may be a substrate for P_4 's effects.

Objective—We investigated the effects of pharmacologically manipulating NMDARs in the VTA for E_2 - and P_4 -facilitated lordosis of hamsters and rats.

Materials and methods—We examined the effect of systemic injections (intraperitoneal; IP) and bilateral infusions to the VTA of the highly specific, competitive NMDAR antagonist, 9-0-6-phosphonomethyl-deca-hydroisoquinoline-3-carboxylic acid (LY235959); 0, 0.3, and 1 mg/kg IP and 0, 1.0, and 10 ng/ μ l intra-VTA), on lordosis of estradiol-primed (E_2 ; 10 μ g) and P_4 -primed (0, 50, 250, or 500 μ g) ovariectomized hamsters and rats.

Results—Intra-VTA administration of the NMDAR antagonist, LY235959, produced similar effects as systemic administration to enhance lordosis, particularly in E_2 -primed rats or hamsters administered moderate P_4 levels (less than 500 μ g). Administration of LY235959 to the substantia nigra did not produce similar effects as intra-VTA infusions for lordosis.

Conclusions—Together, these data suggest that blocking NMDARs in the VTA enhances lordosis of hamsters and rats.

Keywords

GABA_A receptors; Neurosteroids; Nongenomic; Sexual receptivity; Midbrain

Introduction

Progestins have classical or “genomic” actions through binding to intracellular progestin receptors (PRs) as well as rapid, nonclassical (“nongenomic”) actions to mediate sexual behavior of female rodents (as reviewed in Frye et al. 2006a). Progesterone (P_4) has actions to initiate sexual behavior (i.e. lordosis) through binding to estradiol (E_2)-induced intracellular PRs in the ventromedial hypothalamus (VMH; Blaustein 2003; Etgen 1984).

However, duration and intensity of the lordosis response is modulated in part by progestins' actions in the ventral tegmental area (VTA). These actions of progestins in the VTA occur independent of actions at intracellular PRs. First, few E₂-induced PRs have been identified in the VTA (Blaustein 2003; Frye 2001a, b). Second, blocking the few PRs in the VTA does not disrupt P₄-facilitated lordosis (Frye et al. 2000; Frye and Vongher 1999; Whalen et al. 1974). Indeed, similar effects of intravenous progestins to facilitate lordosis are observed in wildtype and PR knockout mice (Frye et al. 2006b; Frye and Vongher 1999; Mani et al. 1996). Third, lordosis of E₂-primed hamsters or rats is rapidly facilitated when free P₄, or P₄ that is conjugated to bovine serum albumin (which does not penetrate neuronal membranes to bind intracellular PRs), is applied to the VTA (Frye and DeBold 1993; Ke and Ramirez 1987, 1990; Frye et al. 1992; Frye and Gardiner 1996a, b). Together, these data support the notion that, in the VMH and VTA, progestins have effects that are PR-dependent and PR-independent, respectively, to modulate lordosis.

In the VTA, P₄ and its metabolites, namely 5 α -pregnan-3 α -ol-20-one (3 α ,5 α -THP), have rapid actions through membrane-bound receptors to mediate the duration and intensity of lordosis. Membrane actions of 3 α ,5 α -THP in the VTA to facilitate lordosis include its positive modulation of γ -aminobutyric acid (GABA)/benzodiazepine receptors (GBRs) (reviewed in Frye et al. 2006a; Majewska et al. 1986). Administration of GBR inhibitors to the VTA attenuates progestin-facilitated lordosis of rats and hamsters (Frye and Vongher 1999; Frye et al. 1993, 2000). Dopamine-like type 1 receptors (D₁) in the VTA are another target of 3 α ,5 α -THP for its rapid effects to mediate lordosis. Administration of the D₁ agonist, SKF38393, or the D₁ antagonist, SCH23390, respectively, increase and decrease progestin-facilitated lordosis of E₂-primed, ovariectomized (ovx) rats and hamsters (Frye et al. 2004; Petralia and Frye 2004; Sumida et al. 2005). Indeed, there is an interaction between effects of 3 α ,5 α -THP at both GBRs and D₁ receptors in the VTA for lordosis. Pretreatment with a GBR antagonist, bicuculline, to the VTA attenuates subsequent facilitation of lordosis by D₁ agonists (Frye et al. 2006a). Together, these data support a role of membrane GABA and D₁ receptors for progestin-facilitated lordosis of rodents.

Although D₁ and GBRs may interact in the VTA to modulate sexual behavior of rodents, *N*-methyl-D-aspartate receptors (NMDARs) in the VTA may also be involved in progestins' actions in the VTA. Glutamate concentrations in the midbrain are greater during behavioral estrus than diestrus and are increased with mating (Frye 2001b). There are NMDARs on neuronal membranes in the VTA (Paquet and Smith 2000). Indeed, GABAergic terminals, which have D₁ receptors, synapse on dopaminergic cell bodies that contain GBRs and NMDARs (Bayer and Pickel 1991; Willick and Kokkinidis 1995). Blocking NMDAR activity by infusions of selective noncompetitive antagonists, (+)-MK-801 hydrogen maleate (MK-801) or dextrorphan, directly to the VTA to E₂- and P₄-primed rats facilitates sexual receptivity over that seen with vehicle infusions (DeBold et al. 2000; Petralia et al. 2007). Although the data suggest that manipulating NMDARs in the VTA may alter lordosis, other studies have demonstrated that MK-801 can have intrinsic rewarding effects. Subcutaneous (SC) injections of MK-801 produce a conditioned place preference among rats (Steinpreis et al. 1995). As such, in the present experiment, we determined the effects of manipulating P₄ and NMDARs in the VTA for lordosis. A competitive NMDAR antagonist, 9-0-6-phosphonomethyl-deca-hydroisoquinoline-3-carboxylic acid (LY235959), was used to test the hypothesis that blocking NMDARs in the VTA would enhance lordosis of ovx, E₂- and/or P₄-primed hamsters and rats. The present experiment used lordosis of both hamsters and rats as an endpoint because hamsters and rats differentially depend upon actions of progestins for lordosis, with hamsters being more heavily reliant upon P₄'s central actions for lordosis than rats are (Frye 2001a, b), which will demonstrate a low level of lordosis following E₂-priming alone. Using this approach, whether effects of manipulating NMDARs in the VTA are related to actions of E₂ or P₄ can begin to be investigated. Although the

present study was not designed to test the interdependence of progesterone and NMDAR action for lordosis, the results of these experiments will inform future studies that will directly examine these interactions.

Materials and methods

These methods were preapproved by the Institutional Animal Care and Use Committee at SUNY Albany.

Animals and housing

Subjects were adult female, LVG hamsters or Long-Evans rats, approximately 55 days of age, which were bred and raised in the animal facility in the Social Sciences Building at SUNY Albany. Original breeder pairs were obtained from Harlan Laboratories (Indianapolis, IA, USA) for hamsters or Taconic Farms (Germantown, NY, USA) for rats. Gonadally intact, sexually experienced male hamsters or rats were used as stimulus males for sexual receptivity testing. Animals were group-housed (hamsters: four per cage, rats: five per cage) in separate temperature-controlled ($22\pm4^{\circ}\text{C}$) rooms in the Laboratory Animal Care Facility and were maintained on a reversed dark/light cycle (lights off between 0800 and 2200 for hamsters and 0800 and 2000 for rats). Rodent chow and water were continuously available for hamsters and rats in their home cages.

Surgery

Surgical procedures were conducted while hamsters were anesthetized with sodium pentobarbital (75 mg/kg intra-peritoneally (IP); Abbott Laboratories, North Chicago, IL, USA) and rats were anesthetized with coadministration of xylazine (12 mg/kg; Bayer, Shawnee Mission, KS, USA) and ketamine hydrochloride (80 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA, USA). All experimental animals were ovx. Ovaries of hamsters were removed via unilateral back incisions and ovaries of rats were removed via bilateral flank incisions. In Experiments 1b and 2b, hamsters and rats were stereotactically implanted with 23-gauge bilateral guide cannulae aimed at the VTA (for hamsters: from bregma $\text{AP} = -2.8$, $\text{ML} = \pm 0.3$, $\text{DV} = -7.0$, according to Morin and Wood 2001; for rats: from bregma $\text{AP} = -5.3$, $\text{ML} = \pm 0.4$, $\text{DV} = -7.0$, according to Paxinos and Watson 1986). All hamsters and rats recovered adequately from surgery and were included in behavioral studies. Surgical recovery was determined by post-surgical neurological testing (i.e. evidence of a righting response, ability to cage climb, response to flank stimulation, good muscle tone and/or weight gain, as per Marshall and Teitlebaum 1974).

E₂ and P₄ administration

For subcutaneous (SC) administration of E₂ and P₄, crystalline steroids were obtained from Steraloids (Newport, RI, USA) and dissolved in corn oil to concentrations of 5 $\mu\text{g}/0.2\text{ cc}$ for E₂ and 50, 250, or 500 $\mu\text{g}/0.2\text{ cc}$ for P₄. All experimental hamsters and rats were administered E₂ at hour 0 and 24 and P₄ at hour 44.

NMDAR antagonist

Pharmacological blockade of NMDARs was achieved with the highly competitive glutamate-site antagonist, LY235959 (Redwine and Trujillo 2003). LY235959 was obtained from Tocris Cookson (Ellisville, MO, USA) and dissolved in sterile saline to reach concentrations of 0.3 or 1.0 mg/kg for IP administration (Experiments 1a and 2a) and 1.0 or 10.0 ng/ μl for central administration to the VTA (Experiments 1b and 2b) at hour 47.5, 30 min prior to behavioral testing. The IP LY235959 regimen has demonstrated effects on antinociceptive tolerance to morphine (Bilsky et al. 1996) and the dosages of 0.3 and 1.0

mg/kg have been used to investigate effects on other motivated behaviors, such as nicotine self-administration (Kenny and Markou 2004).

Drug infusions

Infusions were administered to hamsters and rats under minimal hand-held restraint by the experimenter. Infusions were done with a 1- μ l Hamilton syringe attached to PE-20 tubing connected to a 30-gauge needle (Frye and Vongher 1999, 2001). The rate of infusion was 1.0 μ l/min. To minimize displacement of the infusate, the infusion needle remained in place for 60 s following infusions.

Lordosis test

Lordosis testing of hamsters and rats was done as per methods described in Frye et al. 2006c. Hamsters and rats were tested for sexual behavior in a Plexiglas chamber (50 $\times$ 25 $\times$ 30 cm) with an intact male. The dependent measures were: for hamsters, total duration of lordosis (TLD) following mounts made by a male hamster during a 10-min test and, for rats, the number of times female rats displayed lordosis (lordosis quotient; LQ) following mounts made by a male rat during the 10-min test (max number of mounts was 10).

Activity monitor

At hour 48.5, after hamsters and rats were tested for lordosis, an activity monitor was used to assess motor behavior in some animals in each experimental condition. Subjects were placed in a brightly lit (39 $\times$ 39 $\times$ 30 cm) Digiscan Optical Animal Activity Monitor (Accuscan Instruments, Columbus, OH, USA). The total number of horizontal infrared beam breaks that were made by the subject during the 5-min test was mechanically recorded by the apparatus and then recorded on data sheets by the experimenter (Frye et al. 2006c).

Histology

Following the completion of behavioral testing in Experiments 1b and 2b, animals were intracardially exsanguinated with 0.9% phosphate buffered saline and then perfused with 10% formalin. Brains were post-fixed in 10% formalin overnight followed by storage in 30% sucrose-phosphate buffered saline until they were sliced. Brains were sliced at 40 μ m in a cryostat. The sections were stained with cresyl violet so that infusion locations could be determined by light microscopy. Only data from hamsters ($n=31$) and rats ($n=77$) that received bilateral infusions to the VTA, which could be confirmed by site analyses, were used to determine effects of LY235959 for lordosis.

Procedure

In Experiment 1a, hamsters were E₂-primed at hour 0 and then administered 50, 250, or 500 μ g P₄ SC at hour 44 and IP injections of vehicle or 0.3 or 1.0 mg/kg LY235959 at hour 47.5. In Experiment 1b, hamsters were E₂-primed at hour 0 and then administered 50, 250, or 500 μ g P₄ at hour 44 and intra-VTA infusions of vehicle or 1.0 or 10.0 ng LY235959 at hour 47.5. In Experiment 2a, rats were E₂-primed at hour 0 and then administered vehicle or 50, 250, or 500 μ g P₄ SC at hour 44 and IP injections of vehicle or 0.3 or 1.0 mg/kg LY235959 at hour 47.5. In Experiment 2b, rats were E₂-primed at hour 0 and then administered vehicle or 50, 250, or 500 μ g P₄ at hour 44 and intra-VTA infusions of vehicle or 1.0 or 10.0 ng LY235959 at hour 47.5. Testing occurred at hour 48 in all experiments. Behavioral and site analyses data were collected by observers blind to the hypothesized outcome of the study and/or experimental conditions of the subjects.

Statistical analyses

Two-way analyses of variance (ANOVAs) were used to examine effects of LY235959 drug dosage and P_4 concentration on TLDs and motor behavior of hamsters in Experiments 1a and 1b and LQs and motor behavior of rats in Experiments 2a and 2b. The α level for statistical significance was $p < 0.05$ and a trend was considered when $p = 0.10$. Where appropriate, ANOVAs were followed by protected Fisher's post hoc tests to determine group differences.

Results

Experiment 1a: Systemic LY235959 enhances P_4 -facilitated lordosis of ovx, E_2 -primed hamsters

P_4 increased TLDs of ovx, E_2 -primed hamsters [$F(2,108) = 39.65$, $p < 0.01$] depending on dosing, such that hamsters administered 250 and 500 μg P_4 had significantly higher TLDs than did those administered 50 μg P_4 . LY235959 enhanced lordosis [$F(2,108) = 7.14$, $p < 0.01$] and effects were dose-responsive. Hamsters administered the lower dosage (0.3 mg/kg) of LY235959 had significantly higher TLDs than those administered the higher dosage of LY235959, 1.0 mg/kg, or vehicle IP (see Fig. 1). Hamsters that were administered 0.3 mg/kg LY235959 tended to have reduced motor behavior in the activity monitor compared to hamsters administered 1.0 mg/kg LY235959 [$F(2,79) = 2.69$, $p < 0.07$] (see Table 1).

Experiment 1b: Intra-VTA LY235959 enhances P_4 -facilitated lordosis of ovx, E_2 -primed hamsters

The TLD and activity monitor data from nine hamsters that did not receive bilateral infusions to the VTA are included in Tables 1 and 2. These missed site infusions were proximate to the VTA (i.e. substantia nigra) and demonstrate a pattern of effects that is different from data in hamsters that received bilateral infusions to the VTA. As in Experiment 1a, P_4 increased TLDs [$F(2,107) = 55.47$, $p < 0.01$]. Hamsters administered SC 250 and 500 μg P_4 had significantly higher TLDs than those administered 50 μg P_4 . LY235959 (1.0 ng) significantly increased TLDs compared to vehicle or 10.0 ng LY235959 infusions [$F(2,107) = 3.69$, $p < 0.03$]. There was a tendency for an interaction between these factors that was due to LY235959 increasing lordosis in hamsters that received 250 μg P_4 , rather than 50 or 500 μg P_4 [$F(4,107) = 2.22$, $p < 0.07$] (see Fig. 1). There was also an effect of 10.0 ng LY235959 to the VTA, but not missed sites, to increase motor behavior in the activity monitor [$F(2,107) = 55.47$, $p < 0.01$] (see Table 1).

Experiment 2a: Systemic LY235959 enhances E_2 - and P_4 -facilitated lordosis of ovx rats

P_4 increased LQs of ovx, E_2 -primed rats [$F(3,138) = 4.41$, $p < 0.01$]. LY235959 enhanced LQs [$F(2,138) = 6.60$, $p < 0.01$] compared to vehicle administration. There was an interaction between these variables, such that rats that were administered LY235959 and 0, 50, or 250, but not 500 μg P_4 had increased LQs [$F(6,138) = 2.16$, $p < 0.05$] (see Fig. 2). There were no effects of P_4 or LY235959 for activity monitor motor behavior of rats.

Experiment 2b: Intra-VTA LY235959 enhances E_2 - and P_4 -facilitated lordosis of ovx rats

The LQ and activity monitor data from 42 rats that did not receive bilateral infusions to the VTA are included in Tables 3 and 4. These missed site infusions were proximate to the VTA (i.e. substantia nigra) and demonstrate a pattern of effects that is different from data in rats that received bilateral infusions to the VTA. As in Experiment 2a, P_4 increased LQs [$F(3,144) = 22.72$, $p < 0.01$] compared to vehicle administration. LY235959 significantly increased LQs compared to vehicle infusions [$F(2,144) = 15.69$, $p < 0.03$] (see Fig. 2). There

was a tendency for LY235959 to the VTA to increase motor behavior in the activity monitor [$F(2,112)=2.65$, $p<0.08$] (see Table 3).

Discussion

The present results are consistent with the hypothesis that blocking NMDARs in the VTA enhance sexual receptivity of ovx, E₂- and P₄-primed female rodents. In support, systemic injections of the NMDAR antagonist, LY235959 (0.3 mg/kg), facilitated lordosis of E₂- and P₄-primed hamsters. Intra-VTA administration of LY235959 (1.0 ng) enhanced lordosis of hamsters that were E₂-primed and coadministered 250 µg P₄. A similar pattern of results was observed in rats. In rats that received E₂ alone, or E₂ and 50 or 250 µg P₄, systemic or intra-VTA administration of LY235959 enhanced lordosis. There were some modest effects of LY235959 to enhance motor behavior, as measured by the number of beam breaks made in the activity monitor, in ovx, hormone-primed hamsters and rats. Notably, these behavioral effects of LY235959 were only apparent in hamsters or rats that received LY235959 to the VTA and not to missed sites, such as the substantia nigra. Together, these data suggest that a NMDAR antagonist to the VTA can enhance E₂- and P₄-facilitated lordosis.

The present study confirms and extends previous research on the role of glutamate for sexual behavior of female rodents. Infusions of D,L2-amino-5-phosphonopentonic acid (AP-5) to decrease glutamatergic activity in the mediobasal hypothalamus did not alter lordosis of receptive female rats (McCarthy et al. 1991). Infusions of AP-5 that were more anterior and targeted the preoptic area attenuated lordosis (McCarthy et al. 1991). AP-5 to the VMH enhanced lordosis of rats primed with E₂ alone (Georgescu and Pfaus 2006b). Intrahypothalamic infusions of glutamate agonists, such as NMDA, kainic acid, and potassium chloride, attenuate lordosis of E₂- and/or P₄-primed rats (Georgescu and Pfaus 2006a; Kow et al. 1985). However, infusions of the NMDAR antagonist, MK-801, to the VMH of E₂- and P₄-primed rats and hamsters increases lordosis (Petrallia et al. 2007; DeBold et al. 2000). However, the previous studies on effects of the NMDAR antagonist, MK-801, for lordosis were difficult to interpret because MK-801 can have intrinsic rewarding effects. Systemic administration of MK-801 produces a conditioned place preference (Steinpreis et al. 1995) and infusions of MK-801 to the nucleus accumbens reinforces lever pressing in rats (Carlezon and Wise 1996a, b). Whether manipulating NMDARs in the VTA with MK-801 in this previous study was an effect on the reward system or specific to molecular mechanisms of progestins to facilitate lordosis was unclear. This present study extends present knowledge by examining the effects of a NMDAR antagonist, LY235959, for E₂- and P₄-facilitated lordosis of hamsters and rats, when administered to the VTA. In the present study, infusions of the competitive, highly specific NMDAR antagonist, LY235959, paired with moderate dosages of P₄ (250 µg in hamsters and 50 or 250 µg in rats), enhances lordosis. Indeed, the present data extend these results to suggest that the VTA is one important brain target for NMDAR manipulations to alter lordosis. In support, systemic (which would affect the entire brain) and intra-VTA administration of LY235959 produced similar effects to enhance lordosis. Furthermore, administration of LY235959 to an area outside the VTA, such as the substantia nigra, did not enhance P₄-facilitated lordosis. Similar lordosis-enhancing effects were found in ovx, E₂, and P₄-primed rats that were administered the noncompetitive NMDAR antagonist, MK-801, compared to vehicle, to the VTA (Petrallia et al. 2007). Unlike in the present study, MK-801 when administered to the VTA, increased lordosis at all levels of P₄. LY235959 systemically or to the VTA most robustly increased lordosis at the moderate level of P₄. Therefore, the all-or-none effects of MK-801 to the VTA, compared to the present results that effects of LY235959 are dose-responsive, suggest that the effects in the present study were not solely due to changes in reward and may involve specific effects related to progestin action. This is an important consideration to make given that progestins have

rewarding effects (i.e. produce conditioned place preference), in addition to modulating many other functional effects, including stress responsiveness, learning, anxiety, and ictal processes, through actions in the VTA and mesodopaminergic and limbic connections of the VTA (reviewed in Frye et al. 2006a; Frye 2007). By using lordosis as a bioassay, which requires actions of P_4 (i.e. for hamsters) or is modulated by P_4 (i.e. for rats), future investigations on this topic will reveal the extent to which progestins may have actions involving NMDARs and/or other glutamatergic targets in the VTA.

The present results suggest that, although similar patterns of effects were noted in hamsters and rats, rats may be modestly more sensitive to manipulations of NMDARs. This may be due to differences in reliance on progestins in hamsters and rats. Hamsters require actions of progestins in both the VMH and VTA for lordosis, whereas lordosis can be facilitated by progestins to the VTA of E_2 -primed rats (Frye 2001a, b). As such, lordosis of rats in the present study may have been more sensitive to manipulations of progestins in the VTA. Another reason to investigate mechanisms of progestins in both hamsters and rats is because there are differences in motor demands associated with lordosis of hamsters and rats. Although lordosis of rodents is defined by a rigid immobile stance of the female to allow for mounting and intromission by the male, there are species-specific differences in lordosis and behaviors that precede and follow lordosis. Lordosis of hamsters is characterized by sustained immobility (i.e. for several minutes) before, during, and after mounting by a male. Sexual behavior of rats is characterized by brief lordosis (i.e. for seconds) when the male is mounting. Lordosis of rats is preceded and followed by proceptive behaviors, such as hopping and darting. Considering these differences is important in interpreting the present results in regard to the modest effects of LY235959 for hamster and rat motor behavior in the activity monitor. The highest dosages of LY235959 SC or to the VTA tended to, or significantly, increased motor behavior of hamsters, respectively. No differences were observed in motor behavior of rats administered LY235959 SC and there was a tendency for LY235959 to increase motor activity of rats. Notably, the dose of LY235959 that was most effective in enhancing lordosis did not increase motor behavior. As such, it is not likely that all behavioral effects observed in the present experiment were due to nonspecific effects on general motor behavior.

Although the present experiments begin to address the question of the role of NMDARs as a target of progestins in the VTA, there are several considerations to make regarding interpretations of these results. Additional experiments using NMDAR agonists are necessary to determine if P_4 's effects can be blocked by this manipulation. Indeed, it can be argued that if a behavior can be both enhanced and attenuated by a pharmacological manipulation that a physiologically relevant mechanism is likely being targeted. This will be addressed further in future studies. Another consideration in the interpretation of the present data related to this issue are the effects of LY235959 for lordosis following E_2 -priming alone in ovx rats. The present data demonstrate that LY235959 systemically, or to the VTA, enhance lordosis of E_2 -primed rats. This pattern of results leaves open the possibility that mechanisms of P_4 and actions at NMDARs may be complimentary and/or parallel in the VTA for lordosis. Whether P_4 acts at NMDARs for these effects remains to be investigated and could be addressed by determining the effects of a NMDAR agonist to the VTA. Although these results may suggest that the effects of LY235959 are not progestin-dependent, another interpretation is that E_2 -priming may have enhanced de novo production of the neurosteroid and P_4 metabolite, $3\alpha,5\alpha$ -THP, and LY235959 enhanced the effects of these brain-derived progestins (Cheng and Karavolas 1973). However, these effects were not directly examined in the present study and will need to be addressed in future studies. Additional indirect evidence for the role of progestins in the VTA to underlie the effects observed is related to the established effects of progestins via signal transduction pathways downstream of NMDARs, GBRs, and/or D_1 receptors. D_1 are well-known G-protein

coupled receptors and GBRs can also activate G-proteins and 2nd messengers (Fancsik et al. 2000; Stoof and Kebabian 1984). Indeed, progestins' actions at D₁ and/or GBRs in the VTA require activation of G-proteins, cyclic AMP (cAMP)-dependent protein kinase A, phospholipase C, and/ or PKC for lordosis (Frye et al. 2006b; Petralia and Frye 2004; Petralia et al. 2006; Frye and Walf 2007, papers submitted for publication). Modulating these signal transduction pathways only had effects when administered to E₂- and progestin-primed hamsters and rats, and there was little evidence for effects for E₂-facilitated lordosis. Furthermore, mitogen activated protein kinase (MAPK) is the primary intracellular signaling cascade that is activated by NMDAR phosphorylation (Wang et al. 2007) and a MAPK inhibitor to the VTA attenuates P₄-, but not E₂-, facilitated lordosis of ovx rats (Frye and Walf 2008). Future studies will investigate the signal transduction pathways downstream of NMDARs in the VTA for lordosis. In summary, the present results support the notion that progestins may have actions involving membrane glutamate/NMDARs in the VTA for lordosis of hamsters and rats. Inhibiting NMDARs in the VTA via infusions of the NMDAR antagonist, LY235959, enhanced lordosis at moderate levels, rather than the highest dosage, of P₄ used in the present study.

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Fig. 1.

Top data for hamsters that were E₂- and P₄-primed and received IP administration of vehicle, 0.3 mg/kg LY235959, or 1.0 mg/kg LY235959. *Bottom* data for hamsters that were E₂- and P₄-primed and received infusions of vehicle, 1.0 ng/µl LY235959 or 10.0 ng/µl LY235959 to the VTA. Asterisk indicates a significant effect of vehicle vs. LY235959 ($p < 0.05$). A line and positive symbol above bar indicates a significant effect of 250 or 500 µg P₄ compared to 50 µg of P₄ in hamsters ($p < 0.05$)

Fig. 2. *Top* data for rats that were E₂- and P₄-primed and received IP administration of vehicle, 0.3 mg/kg LY235959 or 1.0 mg/kg LY235959. *Bottom* data for rats that were E₂- and P₄-primed and received infusions of vehicle, 1.0 ng/μl LY235959, or 10.0 ng/μl LY235959 to the VTA. Asterisk indicates a significant effect of vehicle vs. LY235959 ($p < 0.05$). A line and positive symbol above bar indicates a significant effect of rats that were administered 50, 250, or 500 μg P₄ compared to 0 μg of P₄ ($p < 0.05$)

Effects of LY235959 or vehicle SC, infused to the VTA or missed sites of E₂- and P₄-primed hamsters, on motor behavior in the activity monitor (i.e. total number of horizontal beam breaks made)

Table 1

P ₄ dosage	Number of beam breaks									
	IP injection			Infusions to VTA			Infusions to substantia nigra			
	0 mg/kg	0.3 mg/kg	1.0 mg/kg	0 ng/1 µl	1.0 ng/1 µl	10.0 ng/1 µl	0 ng/1 µl	1.0 ng/1 µl	10 ng/1 µl	10 ng/1 µl
50 µg	949 (±99)	908 (±54)	1,061 (±88) ^a	899 (±113)	867 (±82)	1,023 (±71) ^b	1,122 (±119)	1,181 (±178)	1,245 (±11)	
250 µg	804 (±24)	790 (±52)	989 (±77) ^a	712 (±153)	932 (±81)	1,026 (±100) ^b	968 (±0)	806 (±0)	1,023 (±0)	
500 µg	868 (±84)	820 (±38)	839 (±81) ^a	773 (±110)	829 (±55)	1,117 (±120) ^b	1,023 (±0)	1,010 (±117)	1,062 (±10)	

^aIndicates a tendency for SC 1.0 mg/kg LY235959 to increase motor behavior in this task compared to that observed with 0.3 mg/kg LY235959.

^bIndicates that 10.0 ng/µl LY235959 to increase motor behavior in this task compared to vehicle or 1.0 ng/µl LY235959 to the VTA.

Table 2

Total lordosis duration (TLD $\pm$ SEM) of hamsters that received infusions outside of the VTA to the substantia nigra

P ₄ dosage	TLD (s)		
	Infusion to the substantia nigra		
	0 ng/1 μ l	1.0 ng/1 μ l	10 ng/1 μ l
50 μ g	11 ($\pm$ 9)	3 ($\pm$ 3)	0 ($\pm$ 0)
250 μ g	269 ($\pm$ 269)	528 ($\pm$ 0)	572 ($\pm$ 0)
500 μ g	398 ($\pm$ 13)	350 ($\pm$ 106)	164 ($\pm$ 35)

Table 3

Effect of LY235959 or vehicle systemically or infused to the VTA or substantia nigra of E₂- and P₄-primed rats for motor behavior in the activity monitor (i.e. total number of horizontal beam breaks made)

P ₄ dosage	Number of beam breaks									
	IP injection			Infusions to VTA			Infusions to substantia nigra			
	0 mg/kg	0.3 mg/kg	1.0 mg/kg	0 ng/1 µl	1.0 ng/1 µl	10 ng/1 µl	0 ng/1 µl	1.0 ng/1 µl	10 ng/1 µl	10 ng/1 µl
0 µg	544 (±68)	524 (±54)	688 (±81)	532 (±137)	784 (±142) ^a	839 (±158) ^a	949 (±0)	791 (±0)	911 (±181)	
50 µg	483 (±148)	656 (±83)	636 (±89)	516 (±45)	750 (±77) ^a	801 (±90) ^a	326 (±149)	1,166 (±0)	905 (±0)	
250 µg	624 (±54)	494 (±66)	439 (±83)	383 (±24)	911 (±87) ^a	933 (±85) ^a	326 (±149)	1,166 (±0)	905 (±0)	
500 µg	620 (±144)	650 (±65)	493 (±79)	827 (±200)	749 (±84) ^a	687 (±77) ^a	451 (±0)	640 (±87)	907 (±229)	

^a Indicates a tendency for LY235959 to increase motor behavior in this task, compared to vehicle, when administered to the VTA.

Table 4

Lordosis quotients ($LQ \pm SEM$) of rats that received infusions outside of the VTA to the substantia nigra

P ₄ dosage	<u>LQ</u>		
	<u>Infusion to the substantia nigra</u>		
	<u>0 ng/1 μl</u>	<u>1.0 ng/1 μl</u>	<u>10 ng/1 μl</u>
0 μ g	15 (± 6)	44 (± 0)	41 (± 19)
50 μ g	23 (± 9)	20 (± 20)	7 (± 7)
250 μ g	32 (± 8)	25 (± 0)	100 (± 0)
500 μ g	42 (± 10)	36 (± 25)	55 (± 5)