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# Progestins' actions in the VTA to facilitate lordosis involve dopamine-like type 1 and 2 receptors

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**Abstract**

In the ventral tegmental area (VTA), 5α-pregnan-3α-ol-20-one (3α,5α-THP) facilitates lordosis. Whether this involves dopamine type 1 ($D_1$) or dopamine type 2 ($D_2$) receptors is of interest. Ovariectomized (ovx) rats with guide cannulae to the VTA were estradiol ($E_2$) primed and pretested for lordosis. Rats were then infused with the $D_1$ (Experiment 1) or $D_2$ (Experiment 2) antagonists or agonists (0, 100, or 200 ng) to the VTA and were retested. After a second infusion of 3α,5α-THP (0, 100, or 200 ng) or vehicle, rats were tested 10, 60, and 120 min later. In Experiment 3, rats were administered a progestin receptor antagonist, RU38486, systemically or to the VTA 1 h prior to vehicle, SKF38393 and/or 3α,5α-THP infusions. 3α,5α-THP infusions increased lordosis over that seen with $E_2$ priming. The $D_1$ antagonist, SCH23390, attenuated 3α,5α-THP, but not $E_2$-facilitated lordosis. The $D_1$ agonist, SKF38393, augmented 3α,5α-THP, but not $E_2$-facilitated lordosis. The $D_2$ antagonist, sulpiride, had no significant effects on lordosis. The $D_2$ agonist, quinpirole, prevented 3α,5α-THP-facilitated lordosis. RU38486 (subcutaneous) inhibited lordosis, whereas infusions to the VTA decreased lordosis produced by SKF38393 and 3α,5α-THP, but not 3α,5α-THP alone. Thus, 3α,5α-THP's actions in the VTA for lordosis may involve $D_1$ and/or $D_2$ receptors.


*Keywords:* Allopregnanolone; Neurosteroids; Non-genomic; Lordosis; Sexual receptivity

## 1. Introduction

Progestins in the ventral tegmental area (VTA) mediate sexual receptivity of rodents that has been initiated by actions of estradiol ($E_2$) and/or progestins in the ventromedial hypothalamus (VMH). Progestins in the VTA and the VMH are required for lordosis of hamsters (DeBold and Malsbury, 1989). Whereas for rats, progestins in the VTA enhance actions of $E_2$ and/or progesterone (P) in the VMH (Frye and Gardiner, 1996; Pleim et al, 1990, 1991; Rubin and Barfield, 1983a,b). Lesions to the VMH and VTA, respectively, disrupt initiation and maintenance of $E_2$ and P-facilitated lordosis of rodents (Etgen and Barfield, 1986; Malsbury et al., 1977; Mathews and Edwards, 1977; Meisel et al., 1987; Meisel and Sterner, 1990; Muntz et al., 1980). Indeed, the mechanisms through which progestins act in the VMH to initiate sexual receptivity seem to be different from those in the VTA that modulate lordosis once it has commenced.

P's ligand-dependent actions at intracellular progestin receptors in the VMH mediate the onset of lordosis, but P has membrane actions in the VTA to mediate the duration of sexual receptivity. In support, $E_2$-priming increases progestin receptor mRNA (Romano et al., 1989) and synthesis in the VMH (Parsons et al., 1981; 1982a,b), but not the VTA (MacLusky and McEwen, 1980). Blocking progestin receptors, or products of progestin receptor activation, RNA or protein synthesis, in the VMH inhibits, but in the VTA does not disrupt, P-facilitated lordosis (Brown et al., 1987; Frye et al., 2000; Frye and Vongher, 1999a; Mani et al., 1994; Meisel and Pfaff, 1984, 1985; Ogawa and Pfaff, 1998; Rainbow et al., 1982; Whalen et al., 1974). Progestin receptor binding in the VMH (but not the VTA) and lordosis of rats or mice are positively correlated (Frye, 2001a,b; Frye and Vongher, 1999b; Mani et al., 1996; Parsons et al., 1981, 1982a,b; Parsons and Pfaff, 1985). Implants to the VTA, but

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not the VMH, of P conjugated to bovine serum albumin (P:BSA), which does not penetrate neuronal membranes to bind intracellular progestin receptors (Ke and Ramirez, 1987, 1990), rapidly facilitates lordosis of $E_2$-primed rats or hamsters, similar to free P (Frye et al., 1992; Frye and Gardiner, 1996). Thus, an important question is what are the membrane actions by which P in the VTA mediates sexual receptivity of rodents.

In the VTA, formation of 5α-pregnan-3α-ol-20-one (3α,5α-THP), a P metabolite and neurosteroid, and its subsequent actions at membrane $GABA_A$/benzodiazepine receptor complexes (GRBs) are integral for lordosis. 3α,5α-THP is a highly effective positive modulator of GBRs (Wilson, 1996). Interfering with production of, or P's metabolism to, 3α,5α-THP in the VTA inhibits lordosis (Beyer et al., 1999; Frye and Petralia, 2003a,b; Frye and Vongher, 2001). Infusions of GBR agonists or antagonists to the VTA, respectively, increase and decrease progestin-facilitated lordosis (Frye, 2001a,b; Frye et al., 1993). Although 3α,5α-THP in the VTA may facilitate lordosis through membrane actions at GBRs, this does not preclude actions of 3α,5α-THP at other membrane targets.

Some of 3α,5α-THP's actions may involve membrane $D_1$-like ($D_1$ and $D_5$ subtypes) or $D_2$-like ($D_2$, $D_3$, and $D_4$; Tiberi et al., 1991) receptors. First, steroids can alter these receptors. The density of striatal $D_1$ receptors is decreased during proestrus compared to diestrus (Levesque et al., 1989) and with $E_2$ treatment to ovariectomized (ovx) rats (Tonnaer et al., 1989). $D_2$ receptor's affinity is decreased during proestrus compared to diestrus (Di Paolo et al., 1988) and density is reduced with $E_2$ or P treatment to ovx rats (Bazzett and Becker, 1994; Fernandez-Ruiz et al., 1989; Tonnaer et al., 1989). Second, altering $D_1$ receptor activity can influence lordosis. Intravenous or intracerebroventricular $D_1$ agonists enhance, and $D_1$ blockers, inhibit lordosis of ovx, $E_2$-, or $E_2$- and P-primed rats (Apostolakis et al., 1996; Frye et al., 2000; Mani et al., 1994). Third, although systemic administration of $D_2$ agonists can decrease, and antagonists can increase, lordosis of hormone-primed rats (Caggiula et al., 1979; Everitt et al., 1975; Fernandez-Guasti et al., 1987; Foreman and Hall, 1987; Grierson et al., 1988), there are inconsistencies reported. For example, the $D_2$ selective agonist, quinelorane (LY 163502), facilitated lordosis of $E_2$-primed rats but inhibited lordosis of $E_2$- and P-primed rats (Foreman and Hall, 1987). Similarly, sulpiride, a $D_2$-specific antagonist, has been reported to inhibit receptivity of $E_2$- and P-primed rats, facilitate lordosis of $E_2$-primed rats, or have no effect on lordosis of $E_2$-primed rats, or $E_2$- and P-primed hamsters (Grierson et al., 1988; Mani et al., 1994; Meisel et al., 1996). The differential effects on lordosis reported for $D_2$-specific ligands may be related to hormone-milieu and/or divergent effects of drug regimen on activation of presynaptic autoreceptors (which are pharmacologically identical to $D_2$ receptors; Starke et al., 1983; Stoof et al., 1982; Stoof and Kebabian, 1984). Although these data provide some evidence that agonistic actions at $D_1$ may facilitate and at $D_2$ receptors may inhibit lordosis, whether 3α,5α-THP has direct and/or indirect actions at $D_1$ and/or $D_2$ receptors in the VTA to mediate lordosis has not been systematically investigated.

There is some evidence that progestins' actions in the VTA for lordosis may involve $D_1$ and/or $D_2$ receptors. First, $D_1$ and $D_2$ receptors have been localized to the VTA (Boyson et al., 1986; Huang et al., 1992; Mansour et al., 1990). Second, VTA infusions of $D_1$ blockers (SCH23390 or antisense oligonucleotides) or GBR antagonists (picrotoxin, bicuculline, or antisense oligonucleotides for glutamic acid decarboxylase—an enzyme essential for GABA synthesis), but not vehicle, are equally effective at attenuating lordosis of rats and hamsters in behavioral estrous (Frye and Vongher, 1999a). However, whether these effects were due to blocking actions of $E_2$ and/or P was not established.

The present experiment tested the hypothesis that progestins may have actions in the VTA to mediate lordosis in part via $D_1$ and/or $D_2$ receptors. Specifically, we predicted that: If, in the VTA, progestins' actions involve $D_1$ receptors, then antagonism of $D_1$ receptors in the VTA with SCH23390 will attenuate, and activation of $D_1$ receptors in the VTA with SKF38393 will enhance 3α,5α-THP-facilitated lordosis of ovx, $E_2$-primed rats. If progestins' actions in the VTA involve $D_2$ receptors, then antagonism of $D_2$ receptors in the VTA with sulpiride will enhance, and activation of $D_2$ receptors in the VTA with quinpirole will reduce, 3α,5α-THP-facilitated lordosis of ovx, $E_2$-primed rats. Finally, a pilot experiment was conducted to begin to address whether progestin receptor occupancy (in whole brain and/or VTA) influenced effects of 3α,5α-THP and/or SKF38393 to enhance lordosis.

## 2. Method

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

### 2.1. Animals and housing

Subjects were female, Long–Evans rats ($N = 235$), approximately 55 days of age, which were bred and raised in our animal facility from stock obtained from Taconic Farms (Germantown, NY). Gonadally-intact, sexually experienced males were used as stimulus males for sexual receptivity testing. Rats were individually housed in the temperature-controlled (22 ± 4 °C) Laboratory Animal Care Facility and were maintained on a 12:12-h dark/light cycle (lights off between 0800 and 2000 h). Food and water were continuously available for rats in their home cages.

### 2.2. Surgery

Surgical procedures were conducted while rats were anesthetized with Rompun (12 mg/kg; Bayer, Shawnee Mission, KS) and Ketaset (80 mg/kg; Fort Dodge Animal

Health, Fort Dodge, IA). All experimental rats were ovariectomized by bilateral flank incisions and were stereotaxically implanted with 23-gauge, bilateral guide cannulae aimed over the VTA, with coordinates (from bregma AP = − 5.3, ML = ± 0.4, DV = − 7.0), according to Paxinos and Watson (1986). Data of 21 rats were omitted from the analyses because they failed postsurgical neurological testing demonstrated by loss of weight, poor righting response, disregard for flank stimulation, and/or poor muscle tone (Marshall and Teitlebaum, 1974).

### 2.3. Drugs

#### 2.3.1. $D_1$ agents

R(+)-SCH23390 hydrochloride is a highly selective $D_1$ receptor antagonist (Iorio et al., 1983; O'Boyle and Waddington, 1987; Sidhu et al., 1986). It was obtained from Research Biochemicals International (Natick, MA) and dissolved in sterile saline to reach concentrations of 100 or 200 ng in 1 μl. This SCH23390 infusion regimen has demonstrated effects on lordosis within 30 min of infusion, which are sustained for at least 4 h (Frye et al., 2000; Mani et al., 1994) and has also been used to investigate effects of other motivated behaviors (Baldo et al., 2002).

( ± )-SKF38393 hydrochloride, obtained from Sigma (St. Louis, MO), was utilized as a $D_1$ receptor agonist (Ongini et al., 1985). Concentrations of 100 or 200 ng in 1 μl (sterile saline) can alter lordosis within 15 min with effects lasting for at least 3 h (Frye et al., 2000; Mani et al., 1994). Similar regimen of SKF38393 have been used to investigate motor and feeding behavior of rats (Swanson et al., 1997).

#### 2.3.2. $D_2$ agents

(+)-Sulpride, a $D_2$ receptor antagonist (Trabucchi et al., 1975), was obtained from Sigma, and dissolved in sterile saline to reach a concentration of 100 ng in 1 μl. Within 30 min, this sulpiride regimen can block effects of the $D_2$ agonist quinpirole on lordosis (Mani et al., 1994). Sulpiride has also been used to examine the role of $D_2$ receptors in lordosis-mediated conditioned place preference of hamsters (Meisel et al., 1996).

( − )-Quinpirole hydrochloride was utilized as a highly effective $D_2$ receptor agonist (Eilam and Szechtman, 1989; Munro and Kokkinidis, 1997). It was obtained from Sigma and dissolved in sterile saline to produce a concentration of 100 ng in 1 μl. The behavioral effects of this quinpirole regimen occur within 30 min and persist for 3 h (Mani et al., 1994) and similar regimen have been used to assess motor and consummatory behavior (Swanson et al., 1997).

#### 2.3.3. Progestin receptor antagonist

RU38486, a progestin receptor antagonist, was obtained from Sigma, and was administered systemically or to the VTA. For systemic injections, RU38486 was dissolved in sesame oil containing 15% benzyl benzoate and 5% ethyl alcohol to reach a concentration of 5.0 mg in 0.4 ml (Beyer et al., 1995; Vathy et al., 1987, 1989). This systemic RU38486 regimen blocks P-facilitated lordosis for at least 4 h (Beyer et al., 1995; Frye and Vongher, 2001; Vathy et al., 1987, 1989). For intra-VTA administration, removable 30-gauge cannulae inserts were tamped in crystalline RU38486. Inserts were verified as containing 1 μg RU38486, with no drug visible on the outside of the insert, prior to application. This implant regimen blocks progestins receptors for at least 4 h (Frye and Vongher, 1999a).

### 2.4. Infusions

All infusions were administered to rats under minimal hand-held restraint with a 2-μl Hamilton syringe attached to PE-20 tubing connected to a 30-gauge needle (Frye and Vongher, 1999a, 2001). The rate of infusion was 1.0 μl/min. To minimize displacement of the infusate, the infusion needle remained in place for 60 s following infusions.

### 2.5. Hormone-priming

Crystalline 17β-$E_2$ was obtained from Sigma and dissolved in corn oil to concentrations of 10 μg/0.2 cc for subcutaneous administration; rats were primed with 7.5 μg $E_2$. Rather than systemic P priming, which would effect the entire brain, rats received infusions to the VTA of 3α,5α-THP (100 or 200 ng) or β-cyclodextran vehicle. In the VTA, 3α,5α-THP, formed from metabolism of ovarian, adrenal, or centrally produced P, mediates lordosis (Frye, 2001a,b; Frye and Petralia, 2003a,b).

### 2.6. Lordosis test

Rats were repeatedly tested for sexual behavior in a Plexiglas chamber (50 × 25 × 30 cm) with an intact male. The duration of each test was 10 mounts or 10 min, whichever occurred first. Females were affixed with vaginal masks to prevent estrous termination due to vaginocervical stimulation and/or repeated testing (Pfaus et al., 2000). The frequency of lordosis [lordosis quotient (LQ)] and the intensity of lordosis [lordosis rating (LR)], quantified by rating dorsiflexion during lordosis on a scale of 0–3 (Frye et al., 2000), exhibited by experimental female rats were recorded by observers, who were uninformed of the hypothesized outcome of the experiment. From these data, the percentage of occurrence of lordosis in response to each mount [LQ=(no. of lordosis responses/no. of mounts) × 100] and the average LRs were calculated and used for statistical analyses (Hardy and DeBold, 1971). Interrater reliability for these indices of female sexual behavior in our laboratory has a concordance rating of greater than 95%.

### 2.7. Histology

Following the completion of behavioral testing, animals were intracardially exsanguinated with 0.9% phosphate-buff-

ered saline (PBS) and then perfused with 10% formalin. Brains were postfixed in 30% sucrose–PBS and sliced at 40 μm in a cryostat. The sections were stained with cresyl violet so that infusion locations could be determined by light microscopy.

### 2.8. Procedure (Fig. 1)

Experiment 1a: Surgerized rats were randomly assigned to one of three infusion conditions: 100 ng of the $D_1$ antagonist, SCH23390 ($n=18$); 200 ng of SCH23390 ($n=19$); or saline vehicle ($n=11$) (Fig. 1).

Experiment 1b: Another cohort of surgerized rats were randomly assigned to one of three infusion conditions: 100 ng of the $D_1$ agonist, SKF38393 ($n=14$); 200 ng of SKF38393 ($n=16$); or saline vehicle ($n=11$).

Experiment 2a: Ovx rats with guide cannulae aimed to the VTA, from another cohort, were randomly assigned to one of two infusion conditions: 100 ng of the $D_2$ antagonist, sulpiride ($n=14$) or saline vehicle ($n=12$).

Experiment 2b: Surgerized rats from another cohort were randomly assigned to either receive 100 ng of the $D_2$ agonist, quinpirole ($n=14$) or saline vehicle ($n=13$).

For Experiments 1 and 2, each week, rats were injected with $E_2$ at Hour 0, and at Hour 44, they were pretested for lordosis. This was followed by receipt of their assigned infusion condition and testing 30 min later. Rats then received a second infusion of 3α,5α-THP (100 or 200 ng) or vehicle and were tested 10, 60, and 120 min later. Rats were tested once a week for 3 weeks, so that both 3α,5α-THP dosages (100 and 200 ng) and β-cyclodextran vehicle were received.

Experiment 3: Ovx rats with guide cannulae to the VTA were randomly assigned to one of four infusion conditions (vehicle; SKF38393, 100 ng; 3α,5α-THP, 100 ng; or SKF38393 and 3α,5α-THP) and one of three progestin receptor antagonist conditions (vehicle, subcutaneous RU38486, or intra-VTA RU38486). There were six rats in each of these 12 experimental conditions. Rats were primed with $E_2$ at Hour 0. At Hour 43, received either subcutaneous injections of RU38486 (5 mg), intra-VTA inserts filled with RU38486 (1 μg), or no manipulation (control). At Hour 44, rats then received their first infusion of SKF38393 (100 ng) or saline vehicle to the VTA. At Hour 44.5, rats were administered a second infusion of 3α,5α-THP (100 ng) or

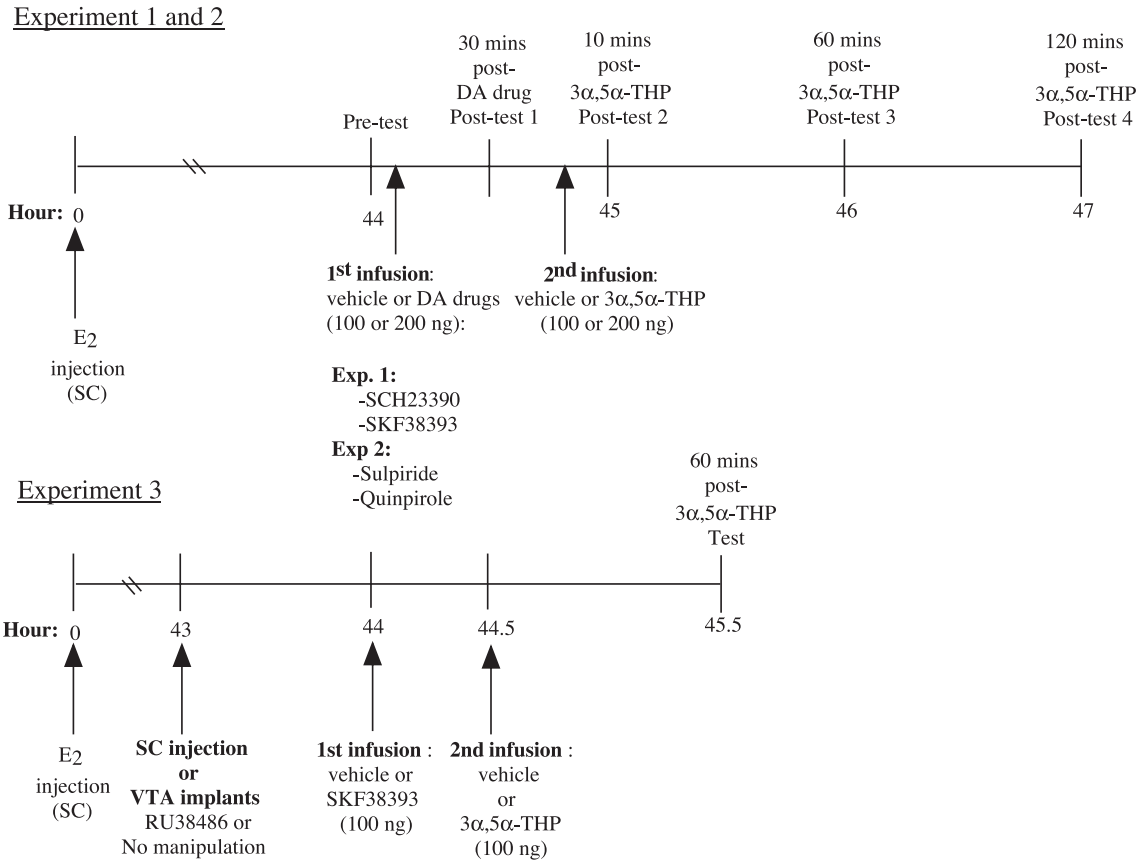


Fig. 1. Schematic of experimental protocol for Experiments 1 and 2 (top) and Experiment 3 (bottom). For Experiments 1 and 2, rats were $E_2$ primed (7.5 μg) at Hour 0 and then pretested for lordosis at Hour 44. Following pretest, rats received first intra-VTA infusion of vehicle, SCH23390, or SKF38393 (Experiment 1) or vehicle, sulpiride, or quinpirole (Experiment 2). In both experiments, rats were then tested 30 min later and, immediately following this test, were infused with vehicle or 3α,5α-THP to the VTA. Rats were tested 10, 60, and 120 min post-3α,5α-THP infusion. For Experiment 3, rats were $E_2$ primed (7.5 μg) at Hour 0; at Hour 43, rats received either subcutaneous or intra-VTA RU38486 or no manipulation (control). At Hour 44, rats received an intra-VTA infusion of vehicle or SKF38393. At Hour 44.5, rats received a second infusion of vehicle or 3α,5α-THP to the VTA and were tested for lordosis at Hour 45.5.

β-cyclodextran vehicle and were tested 60 min later at Hour 45.5.

### 2.9. Statistical analyses

For Experiments 1 and 2, three-way analyses of variance (ANOVAs) were utilized to examine effects of the between-variable (dopamine drug infusion) condition, and the two within variables (3α,5α-THP infusion condition and test time) on LQs and LRs. These overall analyses were followed by one-way ANOVAs, which examined effects of dopamine drug infusions on the average LQs and LRs observed after the second infusion of 3α,5α-THP or vehicle. For Experiment 3, two-way ANOVAs examined effects of infusions and RU38486 conditions. The α level for statistical significance was $P<.05$ and a trend was considered $P<.10$. Where appropriate, ANOVAs were followed by Fisher's *post hoc tests*. Unpaired *t* tests were used to compare differences in mean postinfusion LQs produced by 3α,5α-THP alone or 3α,5α-THP and SKF38393.

There were never any differences observed in the patterns of effects for the quantitative measure of lordosis, LQs, and the qualitative measure of lordosis, LRs. Thus, LR data are not shown.

## 3. Results

### 3.1. Experiment 1a: effects of $D_1$ antagonist, SCH23390 (Table 1; Fig. 2)

The data of six rats ($n=2$/group) were excluded from the analyses because they received infusions to the substantia nigra, rather than the intended site, the VTA. These infusions to the substantia nigra produced a different pattern of effects than infusions to the VTA (Table 1).

When considering the data from 42 rats that received bilateral infusions to the VTA, there was a main effect of the initial infusion to the VTA of the dopamine receptor antagonist, SCH23390, to attenuate lordosis, over that seen with initial infusions of vehicle. SCH23390 infusions to the VTA (11.4 ± 0.8%), compared to vehicle (34.3 ± 2.2%), reduced LQs of rats [$F(2,312)=62.20$, $P<.01$]. Notably, there were no significant differences in LQs produced by the 100 (11.0 ± 0.8%) or 200 ng (11.7 ± 0.8%) dosages of SCH23390. As both similarly reduced LQs compared to vehicle infusion, these groups were combined for follow-up analyses to determine group differences using one-way ANOVAs (Fig. 2, inset).

There was a main effect of the second infusion of 3α,5α-THP to the VTA. 3α,5α-THP infusions increased LQs over that produced by $E_2$-priming alone. Rats administered 3α,5α-THP to the VTA, compared to vehicle administration, had significantly higher LQs [$F(2,312)=20.03$, $P<.01$]. There were no significant differences between LQs produced by 100 (17.6 ± 1.3%) or 200 ng (18.8 ± 1.6%) 3α,5α-THP: both increased LQs compared to vehicle (12.4 ± 0.9%) infusions. Thus, data from rats receiving 100 or 200 ng 3α,5α-THP was combined for the subsequent analyses of group differences using one-way ANOVAs (Fig. 2, inset).

There was a significant interaction between the effects of the first and second infusions [$F(4,312)=11.10$, $P<.0001$]. SCH23390 attenuated 3α,5α-THP-facilitated LQs, but did not significantly alter LQs of rats that were only $E_2$-primed.

There was a main effect of test time [$F(4,312)=41.03$, $P<.01$]. This was due to LQs being greater following the second infusion (19.7 ± 1.9%) than at baseline (11.4 ± 1.1%) or after the first infusion (9.8 ± 1.1%). Initial infusions of neither SCH23390, nor vehicle, influenced LQs compared to those observed on the pretest following $E_2$ priming alone. Within groups, the LQs 10 (18.9 ± 1.8%), 60 (20.9 ± 1.9%), or 120 (20.2 ± 1.9%) min after the second infusion, did not differ. LQs were only increased following the second infusion, if 3α,5α-THP, rather than vehicle, was administered. Thus, the means of the 10-, 60-, and 120-min tests were averaged and group differences were determined with one-way ANOVAs (Fig. 2, inset).

The mean LQs from the 10-, 60-, and 120-min tests were analyzed by a one-way ANOVAs comparing the following conditions: subcutaneous $E_2$ priming only; subcutaneous $E_2$ priming and intra-VTA SCH23390 infusion; subcutaneous $E_2$ priming and intra-VTA 3α,5α-THP infusion; and subcutaneous $E_2$ priming and intra-VTA SCH23390 and 3α,5α-THP infusions. There were significant differences between these groups [$F(3,80)=85.48$, $P<.01$]. *Post hoc* tests revealed that 3α,5α-THP increased LQs of $E_2$-primed rats. Infusions of SCH23390 significantly reduced lordosis of rats receiving 3α,5α-THP infusions, but not $E_2$-priming alone.

Table 1
Effects of infusions of the $D_1$ antagonist, SCH23390, to the substania nigra on LQs (means ± standard error of the mean)

| Infusion condition | | | LQ |
|---|---|---|---|
| *n* | First infusion | Second infusion | Missed sites (substantia nigra) |
| 2 | Vehicle | Vehicle | 9.7 (± 4.6) |
| | Vehicle | 3α,5α-THP 100 | 39.4 (± 4.9) |
| | Vehicle | 3α,5α-THP 200 | 50.0 (± 7.1) |
| 2 | SCH 23390 (100 ng) | Vehicle | 8.8 (± 8.7) |
| | SCH 23390 (100 ng) | 3α,5α-THP 100 | 18.7 (± 5.6) |
| | SCH 23390 (100 ng) | 3α,5α-THP 200 | 7.6 (± 7.1) |
| 2 | SCH 23390 (200 ng) | Vehicle | 8.5 (± 8.7) |
| | SCH 23390 (200 ng) | 3α,5α-THP 100 | 17.3 (± 5.6) |
| | SCH 23390 (200 ng) | 3α,5α-THP 200 | 23.9 (± 8.9) |

### 3.2. Experiment 1b: effects of $D_1$ agonist, SKF38393 (Table 2; Fig. 3)

The data from six rats ($n=3$, vehicle; $n=3$, 200 ng SKF38393) with bilateral infusions to the substantia nigra were excluded from the analyses below (Table 2).

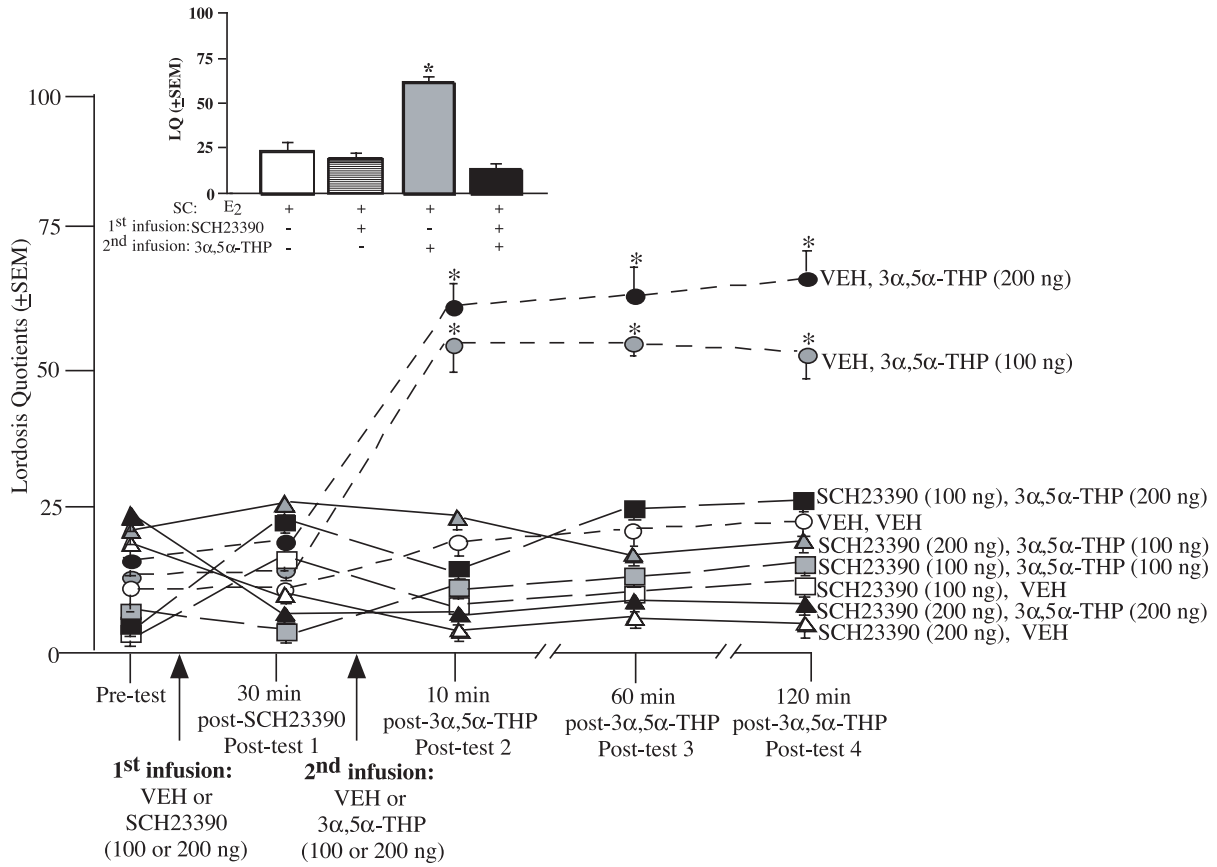


Fig. 2. Mean LQs for rats that received a first infusion of vehicle (VEH; circles), 100 ng SCH23390 (squares), or 200 ng SCH23390 (triangles) and a second infusion of VEH (white), 100 ng 3α,5α-THP (gray), or 200 ng 3α,5α-THP (black) to the VTA. Inset figure depicts mean LQs for the tests 10, 60, and 120 min after a second infusion of 3α,5α-THP or vehicle. * Indicates a significant difference in LQs for groups that received 3α,5α-THP to the VTA, compared to all other groups ($P < .05$).

When considering data from the 35 rats with infusions to the VTA, there was no main effect of an initial infusion of the $D_1$ agonist, SKF38393. LQs produced by the initial infusion of vehicle (34.5 ± 2.4%) were similar to that produced by an initial infusion of 100 (39.5 ± 2.3%) or 200 (38.8 ± 2.1%) ng of the $D_1$ agonist, SKF 38393.

There was a main effect of the second infusion to the VTA [$F(2,256) = 90.78$, $P < .01$]. 3α,5α-THP increased LQs over that produced by $E_2$-priming alone. Rats administered 3α,5α-THP to the VTA, compared to vehicle (18.1 ± 1.3%), had significantly higher LQs following 100 (47.2 ± 2.4%) or 200 ng (48.6 ± 2.1%) 3α,5α-THP infusions.

There was a significant interaction produced by the first and second infusions [$F(4,256) = 3.11$, $P < .02$]. This was attributable to an initial infusion of SKF38393, but not vehicle, enhancing effects of a subsequent 3α,5α-THP infusion on LQs (but having no effects on LQs of rats that were only $E_2$ primed).

There was a main effect of test time [$F(4,256) = 157.14$, $P < .01$]. This was due to LQs being greater following the second infusion (51.5 ± 2.8%) than at baseline (14.7 ± 1.3%) or after the first infusion (20.6 ± 2.0%). Initial infusions of neither SKF38393, nor vehicle, altered LQs compared to those observed on the pretest following $E_2$-priming alone. Within groups, the LQs 10 (48.1 ± 2.8%), 60 (55.7 ± 2.8%), or 120 (50.7 ± 2.9%) min after the second infusion, did not differ. LQs were only increased following the second infusion, if 3α,5α-THP, rather than vehicle, was administered. Thus, the means of the 10-, 60-, and 120-min tests were averaged and group differences were determined using one-way ANOVAs (Fig. 3, inset).

Table 2
Effects of $D_1$ agonist, SKF38393, to the substania nigra on LQs (means ± standard error of the mean)

| Infusion condition | | | LQ |
|---|---|---|---|
| *n* | First infusion | Second infusion | Missed sites (substantia nigra) |
| 3 | Vehicle | Vehicle | 9.0 (± 3.7) |
| | Vehicle | 3α,5α-THP 100 | 27.0 (± 8.0) |
| | Vehicle | 3α,5α-THP 200 | 35.7 (± 10.1) |
| 3 | SKF 38393 (200 ng) | Vehicle | 2.5 (± 2.5) |
| | SKF 38393 (200 ng) | 3α,5α-THP 100 | 57.3 (± 12.0) |
| | SKF 38393 (200 ng) | 3α,5α-THP 200 | 58.7 (± 10.3) |

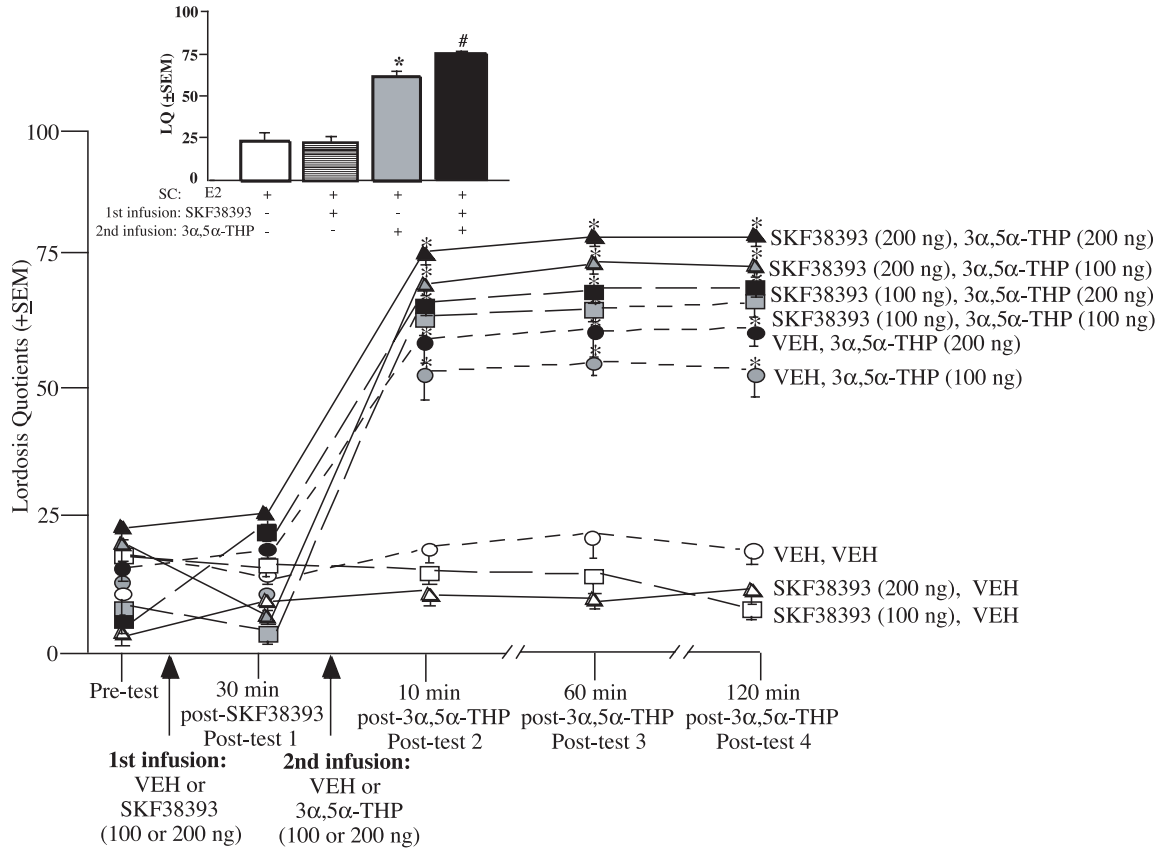


Fig. 3. Mean LQs for rats that received a first infusion of vehicle (VEH; circles), 100 ng SKF38393 (squares), or 200 ng SKF38393 (triangles) and a second infusion of VEH (white), 100 ng 3α,5α-THP (gray), or 200 ng 3α,5α-THP (black) to the VTA. Inset figure depicts mean data of rats after a second infusion of vehicle or 3α,5α-THP. Inset figure depicts mean LQs for the tests 10, 60, and 120 min after a second infusion of 3α,5α-THP or vehicle. * Indicates a significant difference in LQs for groups that received SKF38393 and/or 3α,5α-THP to the VTA, compared to all other groups ($P<.05$). # Indicates that rats infused with SKF38393 and 3α,5α-THP to the VTA tended to have higher LQs than did rats which received vehicle and 3α,5α-THP infusions to the VTA.

One-way ANOVAs revealed a significant difference [$F(3,66)=81.49$, $P<.01$] in LQs among the groups that received subcutaneous $E_2$-priming only, subcutaneous $E_2$ priming and intra-VTA SKF38393 infusion, subcutaneous $E_2$ priming and intra-VTA 3α,5α-THP infusion, or subcutaneous $E_2$ priming and intra-VTA SKF38393 and 3α,5α-THP infusions. *Post hoc* tests revealed that 3α,5α-THP increased LQs of $E_2$-primed rats. Infusions of SKF38393 tended ($P=.06$) to increase LQs of rats that received 3α,5α-THP infusions, but not $E_2$-priming alone.

### 3.3. Experiment 2a: effects of $D_2$ antagonist, sulpiride (Table 3)

The data of six rats ($n=3$/group) were excluded because they received bilateral infusions to the substantia nigra, rather than the VTA (Table 3).

When considering the data of 20 rats that received VTA infusions, there was no main effect of the type of initial infusion. LQs produced by the initial infusion of vehicle (33.9 ± 2.6%) were similar to that produced by an initial infusion of 100 ng of the $D_2$ antagonist, sulpiride (38.7 ± 2.2%).

There was a main effect of the second infusion to the VTA [$F(2,144)=29.95$, $P<.01$]. Rats administered 3α,5α-THP to the VTA, compared to vehicle administration, had significantly higher LQs. Again, both 100 (43.3 ± 2.9%) and 200 ng (44.2 ± 3.0%) infusions of 3α,5α-THP increased LQs compared to vehicle (22.2 ± 2.1%).

There was no significant interaction produced by the first and second infusions.

There was a main effect of test time [$F(4,144)=52.59$, $P<.01$]. This was due to LQs being greater following the

Table 3
Effects of $D_2$ antagonist, sulpiride, to the substania nigra on LQs (means ± standard error of the mean)

| Infusion condition | | | LQ |
|---|---|---|---|
| *n* | First infusion | Second infusion | Missed sites (substantia nigra) |
| 3 | Vehicle | Vehicle | 10.3 (± 3.1) |
| | Vehicle | 3α,5α-THP 100 | 52.8 (± 8.6) |
| | Vehicle | 3α,5α-THP 200 | 37.7 (± 8.9) |
| 3 | Sulpiride (100 ng) | Vehicle | 27.4 (± 4.7) |
| | Sulpiride (100 ng) | 3α,5α-THP 100 | 52.0 (± 8.0) |
| | Sulpiride (100 ng) | 3α,5α-THP 200 | 48.5 (± 6.8) |

second infusion (52.5 ± 3.6%) than at baseline (10.7 ± 1.6%) or after the first infusion (22.2 ± 1.8%). Initial infusions of neither sulpiride, nor vehicle, altered LQs compared to those observed on the pretest following $E_2$-priming alone. Within groups, the LQs 10 (49.4 ± 3.6%), 60 (51.0 ± 3.3%), or 120 (49.5 ± 4.1%) min after the second infusion, did not differ. LQs were only increased following the second infusion, if 3α,5α-THP, rather than vehicle, was administered. Thus, the means of the 10-, 60-, and 120-min tests were averaged and group differences were subsequently determined using one-way ANOVAs (Fig. 4, inset).

One-way ANOVAs comparing average LQs following the second infusion revealed a significant difference among groups [$F(3,36) = 15.41$, $P < .01$]; however, this was due to 3α,5α-THP infusions increasing LQs in the absence (59.1 ± 6.5%) or presence of sulpiride (63.9 ± 4.4%) above that produced by $E_2$-priming alone (25.5 ± 6.7%) or $E_2$ priming and sulpiride to the VTA (27.3 ± 3.3%).

### 3.4. Experiment 2b: effects of $D_2$ agonist, quinpirole (Table 4; Fig. 4)

The data of five rats ($n = 3$, vehicle; $n = 2$, quinpirole) that received bilateral infusions to the substantia nigra, and not the VTA, were excluded (Table 4).

Table 4
Effects of $D_2$ agonist, quinpirole, to the substantia nigra on LQs (means ± standard error of the mean)

| Infusion condition | | | LQ |
|---|---|---|---|
| *n* | First infusion | Second infusion | Missed sites (substantia nigra) |
| 2 | Vehicle | Vehicle | 31.1 (± 4.6) |
| | Vehicle | 3α,5α-THP 100 | 28.0 (± 4.9) |
| | Vehicle | 3α,5α-THP200 | 37.4 (± 7.1) |
| 3 | Quinpirole (100 ng) | Vehicle | 35.4 (± 8.7) |
| | Quinpirole (100 ng) | 3α,5α-THP 100 | 25.9 (± 5.6) |
| | Quinpirole (100 ng) | 3α,5α-THP200 | 54.1 (± 8.9) |

Data from the 22 rats that received VTA infusions, revealed the $D_2$ agonist, quinpirole, did not produce a main effect. LQs produced by an initial infusion of vehicle (32.8 ± 2.2%) or 100 ng of quinpirole (33.5 ± 1.8%) were similar.

There was a main effect of the 3α,5α-THP infusion to the VTA [$F(2,160) = 24.55$, $P < .01$]. Both 100 (39.0 ± 2.5%) and 200 ng (39.2 ± 2.5%) infusions of 3α,5α-THP to the VTA increased LQs, compared to vehicle (21.4 ± 1.8%).

However, there was a significant interaction of quinpirole and 3α,5α-THP [$F(2,160) = 4.81$, $P < .01$]. Quinpirole,

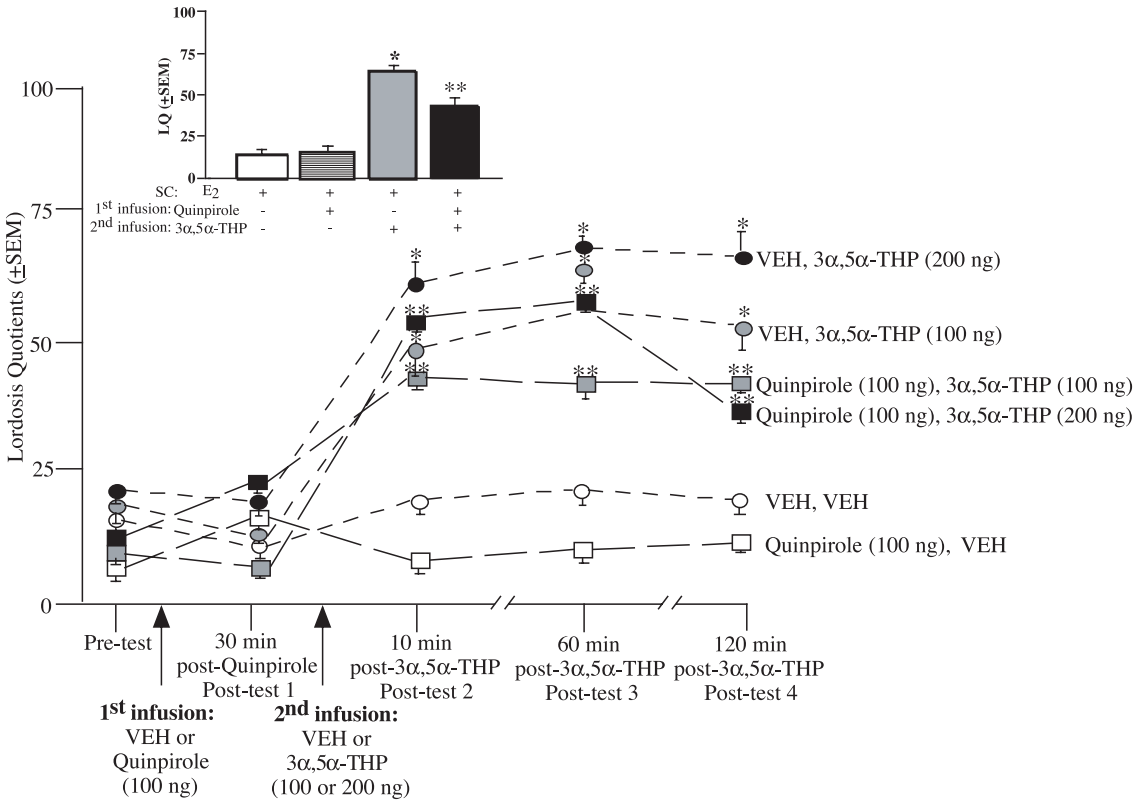


Fig. 4. Mean LQs for rats that received a first infusion of vehicle (VEH; circles) or 100 ng quinpirole (squares) and a second infusion of VEH (white), 100 ng 3α,5α-THP (gray), or 200 ng 3α,5α-THP (black) to the VTA. Inset figure depicts mean data of rats after a second infusion of vehicle or 3α,5α-THP. * Indicates a significant difference in LQs for groups that received quinpirole and/or 3α,5α-THP to the VTA, compared to all other groups ($P < .05$). * * Indicates a significant difference compared vehicle groups ($P < .05$).

compared to vehicle infusions, reduced LQs of rats infused with 100, but not 200 ng, 3α,5α-THP.

There was a main effect of test time [$F(4,160) = 59.77$, $P < .01$]. Again, LQs were higher after the second infusion (44.6 ± 2.8%) than at baseline (9.9 ± 1.5%) or after the first infusions (22.2 ± 2.3%). Within groups, the LQs 10 (38.9 ± 2.4%), 60 (50.7 ± 3.2%), or 120 (44.2 ± 3.0%) min after the second infusion, did not differ.

One-way ANOVAs comparing average LQs following the second infusion revealed a significant difference among groups [$F(3,40) = 80.99$, $P < .01$]. This was attributable to quinpirole, but not vehicle, attenuating 3α,5α-THP-facilitated LQs but having no effect on LQs of rats that were only $E_2$-primed.

### *3.5. Effects of PR antagonist RU38486 and $D_1$ agonist, SKF38393 (Fig. 5)*

There was a main effect of infusion condition [$F(3,60) = 25.06$, $P < .01$]. Rats administered 3α,5α-THP alone (41.5 ± 7.5%) or with SKF38393 (43.9 ± 7.1%) to the VTA had significantly greater LQs compared to those that were $E_2$-primed and received vehicle (9.6 ± 3.6%) or SKF38393 (19.9 ± 3.1%) to the VTA (Fig. 5).

There was a main effect of RU38486 condition [$F(2,60) = 50.02$, $P < .01$]. Rats administered RU38486 systemically (12.6 ± 2.9%) or to the VTA (22.0 ± 4.2%) had significantly lower LQs than rats that were administered vehicle (51.7 ± 6.2%).

There was a significant interaction between infusion and RU38486 condition [$F(6,60) = 6.76$, $P < .01$]. Subcutaneous RU38486 or to the VTA, significantly reduced LQs of $E_2$-primed rats administered vehicle or SKF38393, with or without a second infusion of 3α,5α-THP. Only subcutaneous, and not intra-VTA, RU38486 decreased LQs of rats infused with 3α,5α-THP to the VTA.

## 4. Discussion

The present results are consistent with the hypothesis that agonistic actions at $D_1$ may enhance, and at $D_2$ receptors may inhibit, 3α,5α-THP's effects to facilitate lordosis. In support, infusions of the $D_1$ antagonist, SCH23390, attenuated, and the $D_1$ agonist, SKF38393, enhanced 3α,5α-THP-facilitated lordosis. As well, VTA infusions of the $D_2$ antagonist, quinpirole, reduced effects of 3α,5α-THP infusions to facilitate lordosis. Notably, infusions of the $D_2$ agonist, sulpiride, to the VTA did not influence lordosis. The effects that SCH23390, SKF38393, and quinpirole had on lordosis involved 3α,5α-THP's actions, as these infusions did not produce effects on lordosis due to $E_2$-priming alone. Together, these data support the notion that 3α,5α-THP may have direct and/or indirect actions through $D_1$ and/or $D_2$ receptors to mediate lordosis.

These results confirm previous findings that demonstrate effects of $D_1$ ligands on lordosis and extend them to suggest that $D_1$ receptors in the VTA may be a target for progestins' actions. The present findings include that SKF38393 to the VTA enhanced lordosis of 3α,5α-THP-administered rats, but not those that were $E_2$-primed alone. This pattern of results extends previous findings of effects of SKF38393 on lordosis. For example, intravenous or intracerebroventricular infusions of the $D_1$ agonist, SKF38393, facilitates lordosis of $E_2$-primed rats similar to P priming (Apostolakis et al., 1996; Frye et al., 2000; Mani et al., 1994). The lack of effect of SKF38393 on lordosis of $E_2$-primed rats in the present experiment is likely due to direct administration of SKF38393 to the VTA. Some of these effects of intravenous or intracerebroventricular administration of the $D_1$ agonist may be due to ligand-independent activation of intracellular progestin receptors in the VMH. For example, facilitation of lordosis of rats produced by SKF38393 or P are attenuated by infusions of the $D_1$ antagonist SCH23390, progestin receptor antagonists, or antisense oligonucleotides for progestin re-

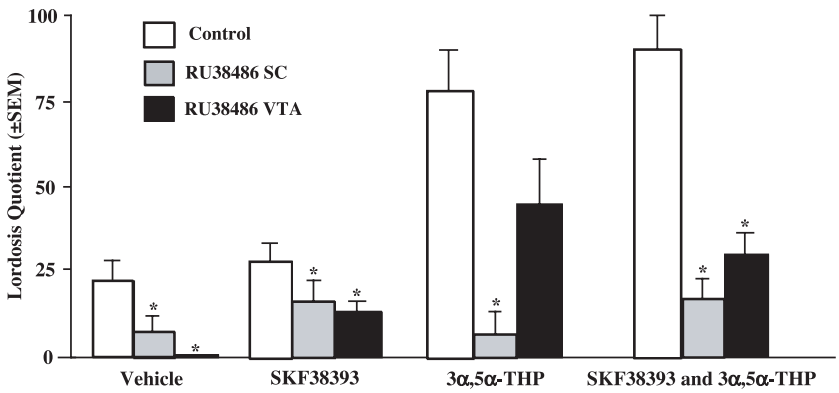


Fig. 5. Mean LQs for $E_2$-primed rats that received vehicle infusions (far left), SKF38393 to the VTA (middle left), 3α,5α-THP to the VTA (middle right), or SKF38393 and 3α,5α-THP to the VTA (far right). The first bar for each condition indicates the control group (white), the second bar shows effects of 5 mg sc RU38468 (gray), and the third bar depicts effects of implants of RU38486 to the VTA (black). * Indicates a significant difference ($P < .05$) from control RU38486 condition for this infusion condition.

ceptor mRNA (Frye et al., 2000; Mani et al., 1994). In addition, progestin receptor null mutation mice, but not their wild-type counterparts, do not show facilitation of lordosis with SKF38393 (Mani et al., 1996). Data from the present report that systemic or intra-VTA administration of a progestin receptor antagonist reduces lordosis of $E_2$-primed rats administered SKF38393 confirm these results, and suggest blocking the $E_2$-induced progestin receptors in the hypothalamus or non-$E_2$-induced progestin receptors in the VTA can attenuate the initiation of SKF38393-facilitated lordosis.

Previous and present findings suggest that 3α,5α-THP-facilitated lordosis may not require actions at intracellular progestin receptors in the VTA. Activation of the many progestin receptors in the VMH is necessary for the initiation of lordosis. Indeed, the systemic RU3848 regimen, which effectively blocks progestin receptors in the VMH, disrupted lordosis, such that manipulations to the VTA did not enhance lordosis. When RU38486 was infused to VTA, an area of the brain with few progestin receptors (Blaustein et al., 1988; Frye, 2001a,b; Frye and Vongher, 1999a; MacLusky and McEwen, 1980; Munn et al., 1983; Turcotte and Blaustein, 1993; Warembourg et al., 1986), lordosis was significantly reduced in all groups except 3α,5α-THP infusions to the VTA. In this latter condition, RU38486 to the VTA decreased lordosis of 3α,5α-THP-infused rats, albeit this difference was not significantly different from 3α,5α-THP-infused control rats. In physiological concentrations, as were utilized in this experiment, 3α,5α-THP is devoid of activity at progestin receptors (Rupprecht et al., 1993). Perhaps, infusions of 3α,5α-THP produced acute supraphysiological concentrations which altered progestin receptors, and/or glucocorticoid receptors (Patchev and Almeida, 1996), such as RU38486, contributed to the nonsignificant reduction in lordosis. Another intriguing aspect of these findings are the results which suggest that SKF38393 may enhance 3α,5α-THP-facilitated lordosis, in part through actions at the few non-$E_2$-induced intracellular progrestin receptors in the VTA. However, before reaching these conclusions, future experiments will need to investigate how the present manipulations affected progestin and/or glucocorticoid receptors in the VTA, VMH, and elsewhere in the brain, and contributed to the present findings.

Infusions of SKF38393 to the VTA increased 3α-5α-THP-facilitated lordosis in this study, suggesting that activating $D_1$ receptors in the VTA may influence lordosis. However, SKF38393 is a partial agonist that elicits 50% or less of dopamine's maximal response (Lovenberg et al., 1989; Mottola et al., 1996; Setler et al., 1978). Additionally, SKF38393 has greater effects on dopamine activity depending upon basal dopamine levels and the density of $D_1$ receptors (Watts et al., 1995). Our data that SKF38393 enhanced 3α,5α-THP-facilitated lordosis, and the opposite effects were observed with the $D_1$ antagonist, SCH23390, imply that SKF38393 exerted agonist-like actions.

$D_1$ receptor agonists may have actions in the VTA to facilitate lordosis via second messenger systems. $D_1$ receptors are positively coupled to adenyl cyclase. Both P and dopamine modulate adenosine 3′,5′-monophosphate (cAMP; Collado et al., 1985; Etgen et al., 2001; Frye, 2001a). Up-regulation of cAMP can enhance lordosis (Beyer et al., 1981; Collado et al., 1985; Uphouse et al., 2000). Thus, activation of $D_1$ receptors in the VTA may increase lordosis of rats by increasing cAMP, protein kinase A, and/or phosphorylation of proteins that are integral for mating.

$D_2$ receptors in the VTA may also be involved in the inhibitory actions of $D_2$ receptor agonists on lordosis. Peripheral administration of $D_2$ agonists, bromocriptine, quinpirole, or LY163502, has a suppressive effect on lordosis (Ahlenius, 1993; Everitt et al., 1975; Fernandez-Guasti et al., 1987; Foreman and Hall, 1987; Grierson et al., 1988). Systemic administration of olanzapine, which blocks about 80% of $D_2$ receptors (Kapur et al., 1998) and enhances 3α,5α-THP levels (Frye and Seliga, 2002; Marx et al., 2000, 2003), facilitates lordosis of $E_2$-primed rats (Frye and Seliga, 2002). Evidence for actions at $D_2$ receptors in the VTA are as follows. There is a moderate density of $D_2$ binding, and high levels of $D_2$ receptor mRNA in the VTA (Mansour et al., 1990; Weiner et al., 1991). Intra-VTA or peripheral olanzapine, a $D_2$ antagonist, similarly enhances lordosis and 3α,5α-THP levels (Frye and Seliga, 2002, 2003). Results from the present experiment that VTA infusions of a $D_2$ agonist, quinpirole (100 ng), attenuate 3α,5α-THP-facilitated lordosis (albeit not as robustly as do infusions of the $D_1$ antagonist, SCH23390) also support

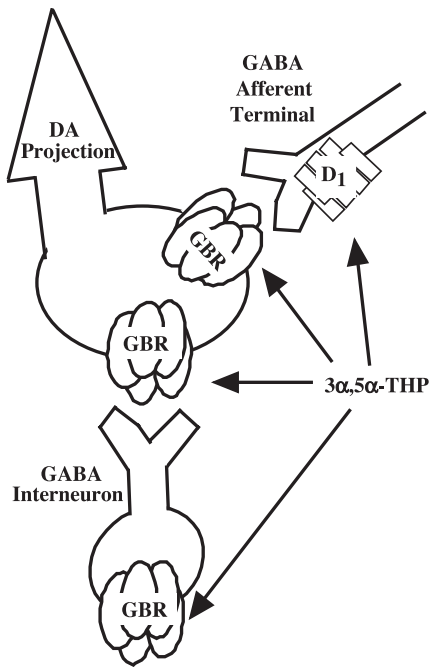


Fig. 6. Putative mechanisms for progestin-facilitated lordosis may involve actions of 3α,5α-THP at $D_1$, $D_2$, and/or γ-aminobutyric $(GABA)_A$/benzodiazepine receptors (GBRs). 3α,5α-THP's effects in the VTA to enhance lordosis may be through dopamine (DA), which is increased by progestins' actions at GBRs to prolong the opening of the chloride channel, and/or by progestins acting on $D_1$ receptors to increase GABA release.

inhibitory actions via $D_2$ receptors in the VTA. These findings are consistent with previous studies that showed that activation of presynaptic $D_2$ autoreceptors can enhance lordosis and phasic release of dopamine (Grierson et al., 1988; O'Connor and Brown, 1982; Starke et al., 1983; Stoof et al., 1982; Stoof and Kebabian, 1984). However, sulpiride, a $D_2$ antagonist, did not enhance 3α,5α-THP-facilitated lordosis, despite previous findings that antagonizing $D_2$ autoreceptors inhibits sexual receptivity (Grierson et al., 1988). Together, these data suggest that agonistic actions at $D_2$ receptors in the VTA can inhibit progestin-facilitated lordosis.

Given that activating $D_1$ and $D_2$ receptors in the VTA, respectively, amplifies and prevents 3α,5α-THP-facilitated lordosis, an important question is how might these actions of progestins relate to their actions at γ-aminobutyric benzodiazepine receptors. There are $D_1$ receptors on GABA afferents in the VTA and enhancing their activity increases GABA release (Cameron and Williams, 1993). Increasing GABA in the VTA increases dopamine secretion (Klitenick et al., 1992), which is known to occur in the VTA and nucleus accumbens concomittant with mating and/or other motivated behaviors (Becker et al., 2001; Berridge et al., 1997; Frye, 2001a; Jenkins and Becker, 2003; Kohlert and Meisel, 1999; Martinez and Paredes, 2001; Meisel et al., 1993; Mermelstein and Becker, 1995; Paredes and Alonso, 1997; Paredes and Vazquez, 1999; Xiao and Becker, 1994). Thus, progestins may have complementary actions in the VTA via GBRs and $D_1$ and/or $D_2$ receptors to increase dopamine levels. Progestins may have redundant actions via GBRs to prolong the opening of the chloride channel or via $D_1$ receptors to increase GABA release (see Fig. 6). The precise processes that progestins actions at $D_1$ and/or $D_2$ receptors provoke, and the underlying neural circuitry associated with the substrates in the VTA through which progestins facilitate sexual receptivity, is an ongoing subject of investigation in our laboratory.

In summary, the present results support the notion that progestins can have actions at membrane dopamine receptors in the VTA to influence 3α-5α-THP-facilitated lordosis. The $D_1$ antagonist, SCH23390, attenuated 3α,5α-THP-facilitated lordosis. The $D_1$ agonist, SKF38393, enhanced 3α,5α-THP-facilitated lordosis. The $D_2$ agonist, quinpirole, prevented 3α,5α-THP-facilitated lordosis. All of the effects were specific to 3α,5α-THP, as neither SCH23390, SKF38393, nor quinpirole had effects on lordosis of $E_2$-primed rats. Together, these data suggest that in the VTA, 3α,5α-THP's actions for lordosis involve $D_1$ and $D_2$ receptors.

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