Published in final edited form as:

Psychopharmacology (Berl). 2011 January ; 213(1): 93–103. doi:10.1007/s00213-010-2016-3.

Effects of neurosteroid actions at *N*-methyl-D-aspartate and GABAA receptors in the midbrain ventral tegmental area for anxiety-like and mating behavior of female rats

Cheryl A. Frye and

Department of Psychology, The University at Albany–SUNY, Life Sciences Research Building 01058, 1400 Washington Avenue, Albany, NY 12222, USA. Department of Biological Sciences, The University at Albany–SUNY, Albany, NY, USA. Center for Neuroscience Research, The University at Albany–SUNY, Albany, NY, USA. Center for Life Sciences Research, The University at Albany–SUNY, Albany, NY, USA

Jason J. Paris

Department of Psychology, The University at Albany–SUNY, Life Sciences Research Building 01058, 1400 Washington Avenue, Albany, NY 12222, USA

Cheryl A. Frye: cafrye@albany.edu

Abstract

Rationale—In the midbrain ventral tegmental area (VTA), actions of neurosteroids, such as the progesterone metabolite, 5 α -pregnan-3 α -ol-20-one (3 α ,5 α -THP), can facilitate mating and influence stress-related processes. Some actions of 3 α ,5 α -THP may occur via positive modulation of GABA_A receptors (GBRs), or negative modulation of *N*-methyl-D-aspartate receptors (NMDARs), to influence anxiety-like behavior; but this is not known.

Objectives—We aimed to assess the role that neurosteroids and stress factors play on intra-VTA NMDAR- and/or GBR-mediated anxiety-like and mating behavior.

Methods—Estradiol-primed, ovariectomized rats, which were partially or completely adrenalectomized (ADX), received infusions of vehicle, an NMDAR blocker (MK-801; 200 ng), or a GBR antagonist (bicuculline, 100 ng) to the VTA. Rats then received intra-VTA vehicle or a neurosteroidogenesis enhancer (N,N-Dihexyl-2-(4-fluorophenyl)indole-3-acetamide, FGIN 1-27, 5 μ g) and anxiety-like and sexual behavior was assessed.

Results—Complete, compared to partial, ADX significantly reduced open arm exploration on an elevated plus maze, the proportion of females that engaged in mating, lordosis quotients, pacing of sexual contacts, and defensive aggression towards a sexually vigorous male. Intra-VTA MK-801 enhanced open arm investigation and the proportion of females that engaged in mating. Infusions of either, MK-801 or FGIN 1-27, enhanced lordosis and, when co-administered, FGIN 1-27 attenuated MK-801's lordosis-enhancing effects. Intra-VTA infusions of bicuculline, prior to FGIN 1-27, blocked FGIN 1-27's effects to enhance lordosis.

Conclusions—Together, these data suggest that reduced NMDAR activity in the VTA may influence motivation to explore and engage in sexual behavior. These data suggest that neurosteroid actions at NMDARs and GBRs in the VTA are important for exploration and/or sexual behavior.

Keywords

Allopregnanolone; Anxiety; Corticosterone; Dizocilpine; Hypothalamic–pituitary–adrenal axis; Lordosis; Progesterone

Introduction

We have investigated the midbrain ventral tegmental area (VTA) for its sensitivity to the effects of steroid hormones on sexual behavior. In female rats, progesterone (P) secretion from peripheral endocrine glands (ovaries and adrenals) is enhanced cyclically. Peripheral P can readily be metabolized in brain to form dihydroprogesterone (DHP) and, subsequently, 3 α ,5 α -pregnan-3 α -ol-20-one (3 α ,5 α -THP, a.k.a. allopregnanolone). Unlike P and DHP, which act at intracellular progesterone receptors, 3 α ,5 α -THP acts at neurotransmitter targets, such as excitatory *N*-methyl-D-aspartate receptors (NMDARs; Johansson and Le Grevès 2005) and inhibitory GABA_A receptors (GBRs; Majewska et al. 1986). Infusing P or 3 α ,5 α -THP to the VTA of hormone-primed rats or hamsters rapidly facilitates lordosis (the stereotypical posture female rodents engage in to copulate) and pharmacologically blocking intra-VTA NMDARs enhances (Petralia et al. 2007), whereas blockade of GBRs reduces (Frye and Vongher 1999), lordosis among receptive rats and hamsters. Thus, actions of 3 α ,5 α -THP at non-traditional steroid targets mediate lordosis.

In addition to metabolism from peripheral P, 3 α ,5 α -THP can also be formed *de novo* in the brain within glial and neuronal cells. There exists a dynamic relationship between central biosynthesis and mating, wherein, 3 α ,5 α -THP is rapidly synthesized in midbrain, hippocampus, prefrontal cortex, and diencephalon of mated female rats (Frye et al. 2007b) and blockade of this formation attenuates mating (Frye et al. 2009). Whether actions at NMDARs and/or GBRs are required for these effects is not known.

Mating is a complex act that encompasses a suite of reproductively relevant behaviors including affective processes. Rodent mating is characterized by (1) an appetitive phase, wherein female rats demonstrate proceptive cues (such as hopping, darting, ear wiggling) to solicit males, (2) a consummatory phase, wherein females engage in lordosis upon mounting by a male, and (3) a post-consummatory phase, wherein there is a refractory period (Beach 1976). Approach/avoidance between mounts allows females to temporally “pace” their sexual contacts and this aspect of mating is associated with neurosteroidogenesis (Frye et al. 2007b). For this behavioral scheme to occur, natural responses to anxiogenic stimuli must be suppressed and this may involve actions of centrally derived 3 α ,5 α -THP on intra-VTA NMDARs and/or GBRs.

In addition to its role in mating, the VTA is a critical region for response to stimuli that are considered rewarding for their properties to condition a place preference. In particular, the VTA is involved in natural reward states, such as engagement in mating (Frye et al. 2009), aggression (Anstrom et al. 2009), feeding (Trojnar and Staszewska 1994), and/or sucrose consumption (Shimura et al. 2002), which can condition a place preference (Martínez and Paredes 2001; Perks and Clifton 1997). While, the mechanisms that underlie these effects are not known, an important component appears to involve glutamate receptors. Like the hippocampus, the VTA is characterized by plastic changes in potentiation of transmission at glutamatergic synapses (Bonci and Malenka 1999). However, the importance of intra-VTA NMDARs for a naturally rewarding stimulus, such as mating, is not well-understood.

We aimed to assess the mechanisms by which central neurosteroid formation in the VTA may influence engagement in paced mating. Given that stress activation can enhance 3 α ,5 α -THP formation in the brain (Purdy et al. 1991), partly via peripheral gland production,

female rats were ovariectomized and adrenalectomized (OVX/ADX) to assess the specificity of these effects on neurosteroidogenesis. In the past, we have conducted these pharmacological interventions among intact proestrous and OVX rats that are adrenally intact (Frye and Paris 2009; Petralia et al. 2007; Frye et al. 2006a, 2009) and have included a small subset of these groups for points of reference. From experience, we anticipated that not all rats that underwent ADX would have the adrenals completely extirpated, as even a few remaining adrenal cells will continue to produce glucocorticoids. OVX rats with complete, or partial, ADX were hormone-primed and infused with vehicle, an NMDAR blocker (MK-801) or a GBR blocker (bicuculline) to the VTA. Rats then received subsequent infusions of a compound that enhances neurosteroidogenesis (N,N-Dihexyl-2-(4-fluorophenyl)indole-3-acetamide, FGIN 1-27) or vehicle. Rats were assessed for performance in the elevated plus maze, social interaction, and paced mating tasks. We anticipated that: (1) complete ADX would reduce exploratory and mating behavior compared to incomplete ADX, (2) MK-801 or FGIN 1-27 would enhance, and bicuculline would reduce, exploration and engagement in mating, and (3) enhancing effects of FGIN 1-27 would be attenuated by blockade of NMDARs or GBRs.

Materials and methods

These methods were approved by the Institutional Animal Care and Use Committee at The University at Albany–SUNY.

Subjects and housing

Adult, Long–Evans female rats ($n=174$), approximately 50–60 days of age, were bred in the Life Sciences Laboratory Animal Care Facility at The University at Albany–SUNY (original stock: Charles River, Raleigh, NC, USA). Rats were group-housed in polycarbonate cages with woodchip bedding (45×24×21 cm) in a temperature-(21±1°C) and humidity- (50±5%) controlled room in the Laboratory Animal Care Facility. Rats were maintained on a 12:12 h reversed light cycle (lights off at 0800 hours) with continuous access to Purina Rat Chow and tap water in their home cages.

Surgical manipulations

Rats were stereotaxically implanted with bilateral guide cannulae aimed at the medial aspect of the VTA (from bregma: AP=−5.3, ML=±0.4, DV=−7.0; Paxinos and Watson 1986) under xylazine (12 mg/kg) and ketamine (70 mg/kg) anesthesia per prior methods (Frye et al. 2009). Immediately following stereotaxic surgery, some rats were OVX and ADX ($n=152$), a small control group of rats were OVX and left adrenally intact ($n=13$), and another small control group of rats were left gonadally and adrenally intact ($n=9$). For OVX/ADX, dorsal incisions were made between the ribs and hip, so that the ovaries and the adrenals could be isolated. The ovaries were ligated and removed, and the adrenals were isolated and removed. As ADX rats are sodium deficient, all rats received a bottle of 0.9% saline and a bottle of tap water in their home cages. Following surgery and prior to testing, animals were monitored for loss of weight, righting response, flank stimulation response, and/or muscle tone (Marshall and Teitelbaum 1974). No rats failed these assessments in the present study. All rats that underwent ADX were screened for complete ADX via post hoc assessment of corticosterone in plasma (methods below). Rats with concentrations of corticosterone below baseline levels (<1 µg/dl) were considered to be completely ADX ($n=79$). Rats with concentrations of corticosterone above baseline levels (>1 µg/dl) were considered to be partially ADX ($n=73$).

Central infusions

The NMDAR blocker, MK-801 hydrogen maleate, was obtained from Sigma Chemical Co. (St. Louis, MO) and was diluted to a concentration of 200 ng/ μ l in sterile saline. MK-801, is a long-lasting non-competitive antagonist that acts in the NMDAR channel pore, where it can be trapped, to block opening (Dravid et al. 2007). This regimen facilitates lordosis of E- and/or P-primed rodents (Petrulia et al. 2007).

The GBR blocker, bicuculline, was also obtained from Sigma Chemical Co. (St. Louis, MO) and dissolved in sterile saline to a concentration of 100 ng/ μ l for intra-VTA infusions. Bicuculline is a non-competitive antagonist that inhibits the GABA_A ion channel (Ueno et al. 1997). This regimen attenuates lordosis of naturally cycling, E- and/or P-primed rodents (Frye and Paris 2009; Frye et al. 2006b).

The neurosteroidogenesis enhancer, FGIN 1-27, is an agonist of the 18 kDa translocator protein (Romeo et al. 1992). FGIN 1-27 was obtained from Alexis Biomedicals (San Diego, CA) and was dissolved in saline with 2% methanol (MeOH) to a concentration of 5 μ g/ μ l. We have previously demonstrated this regimen to enhance lordosis of naturally cycling rats or OVX rats and hamsters primed with E and/or P (Frye et al. 2009; Petrulia and Frye 2005).

Procedure

All surgitized rats were allowed 7 days for surgical recovery and washout of endogenous hormones. Forty-four hours prior to testing, rats were primed with E (10 μ g, SC), as this regimen facilitates sexual behavior of OVX rats (Frye et al. 2008b). The small control group of OVX rats that were adrenally replete were administered progesterone (50 μ g, SC), 3 h prior to testing, so that they would be commensurate to the gonadally and adrenally intact proestrous rats in terms of circulating hormone concentrations per previous methods (Petrulia et al. 2007). The small control group of intact rats had their estrous cycle phase determined via vaginal lavage and inspection of epithelium under a light microscope per previous methods (Frye et al. 2000). Rats were randomly assigned to receive a bilateral infusion (1 μ l) of saline vehicle, MK-801 (200 ng per cannula), or bicuculline (100 ng per cannula) to the midbrain VTA, 30 min prior to testing. OVX/ADX rats were then randomly assigned to receive a subsequent infusion of vehicle (2% MeOH in saline), or FGIN 1-27 (100 ng per cannula) to the VTA, 10 min prior to testing. Prior investigations aimed at assessing the dose–response of MK-801 (Petrulia et al. 2007), bicuculline (Frye et al. 1993), or FGIN 1-27 (Frye and Petrulia 2003a, b) for effects on lordosis, when infused to the VTA of rodents, have determined these regimen to be optimal. We, and others, have demonstrated pharmacological effects of these compounds on behavior for up to 90 min (Frye and Paris 2009; Frye et al. 2009; Li et al. 2009). As such, there were six conditions in the partial ADX group: vehicle/vehicle ($n=12$), vehicle/FGIN 1-27 ($n=13$), MK-801/vehicle ($n=18$), MK-801/FGIN 1-27 ($n=9$), bicuculline/vehicle ($n=12$), and bicuculline/FGIN 1-27 ($n=9$). As well, there were six conditions in the complete ADX group: vehicle/vehicle ($n=13$), vehicle/FGIN 1-27 ($n=14$), MK-801/vehicle ($n=18$), MK-801/FGIN 1-27 ($n=8$), bicuculline/vehicle ($n=15$), and bicuculline/FGIN 1-27 ($n=11$). Following infusions, rats were tested in all tasks described below, during a single testing session.

Behavioral testing

All rats were tested in one task after another, without delay. This was done to minimize carryover effects, wherein, more intense anxiety exposure or social stimulation can alter behavioral and/or neuroendocrine responding in following tasks. In support, we have found that engagement in paced mating significantly alters neuroendocrine milieu among female rats (Frye et al. 2007b). As such, rats were assessed in tasks with the most intense social-anxiety components (such as social interaction and paced mating) last. Prior to testing, all

rats were placed in an open field apparatus (76×57×35 cm) that consisted of a 48-square grid floor (6×8 squares, 9.5 cm/side) for 5 min to habituate subjects to the testing room and assess general motor behavior (Frye et al. 2000).

Elevated plus maze—The elevated plus maze was conducted as previously described (Frye et al. 2000). Rats were placed in the center of the maze, and the amount of time spent on (and number of entries into) open or closed arms (each 49 cm long, 10 cm wide; elevated 50 cm from the ground) were recorded for 5 min. Open arm time and total arm entries indicate exploratory/anti-anxiety and motor behavior, respectively.

Social interaction—Social interaction was conducted as previously described (Frye et al. 2000). Social interaction of an experimental rat with an OVX conspecific rat was indicated in a 5-min task by crawling over or under, sniffing, following with contact, genital investigation, tumbling, boxing, or grooming within an open field apparatus. Duration of interaction indicates social exploration.

Paced mating—Paced mating was conducted per previous methods (Erskine 1985) in an apparatus (37.5×75×30 cm) that was equally divided by a Plexiglass partition. An experimental female had access to both sides of the pacing chamber, while a stimulus male was confined to one side for a 15-min test period. For statistical analyses, quotient values were calculated for lordosis [(frequency of mounts responded to with lordosis/frequency of mounts received) × 100], defensive aggression (vocalizing, threatening, attacking) [(frequency of mounts responded to with aggression/frequency of mounts received) × 100], and pacing of sexual contacts which was indicated by exiting the male chamber following mounts [(frequency of exits following mount/frequency of mounts received) × 100]. Pacing is an index of motivation to engage in sexual stimuli. The proportion of rats engaging in mating is also calculated. While all rats were mounted (Table 1), rats were considered not to engage in mating if they did not display lordosis to male attempts at intromission. Data from all rats is included in analyses.

Tissue collection for radioimmunoassay

Immediately after testing, rats were decapitated and trunk blood and whole brains were collected. Trunk blood was centrifuged at 3,000×g for 10 min, and serum was stored at −20°C. Whole brains were stored at −80°C. Tissues were assessed for concentrations of circulating and central corticosterone (plasma only), E, P, DHP, and 3α,5α-THP. Prior to dissection, brains were thawed on ice and tissues were visually inspected for infusion locations congruent with a miss (Fig. 1, left arrow) or a hit (Fig. 1, right arrow) to the midbrain VTA, per previously reported methods (Frye and Paris 2009). Infusions to nearby missed sites (including substantia nigra and central gray) do not evoke consistent changes in anxiety-like and/or sexual behavior (Frye et al. 2008b). Twenty-two brains (*n*=10 partial ADX; *n*=12 complete ADX) had infusions to sites other than the VTA and were excluded from analyses. Midbrain, hippocampus, cortex, and diencephalon were dissected, and plasma was extracted as described (Frye et al. 2007b).

Radioimmunoassay for steroid hormones

Concentrations of corticosterone, P, DHP, 3α,5α-THP, and E were assessed using previously reported methods (Frye et al. 2008b). Standard curves ranged from 10–4,000 pg for corticosterone, 50–8,000 pg for P, DHP, and 3α,5α-THP, and 12.5–1,000 pg for E. The inter- and intra-assay coefficients of variance were: corticosterone 0.04 and 0.08, P 0.10 and 0.11, DHP 0.10 and 0.04, 3α,5α-THP 0.10 and 0.13, and E 0.06 and 0.06.

Statistical analyses

Among OVX/ADX rats, three-way analyses of variance (ANOVAs) were utilized to assess behavioral and neuroendocrine effects of the following independent variables: (1) adrenalectomy condition (partial, complete), (2) intra-VTA blocker condition (vehicle, MK-801, bicuculline), and (3) subsequent intra-VTA FGIN 1-27 condition (vehicle, FGIN 1-27). Group differences for main effects were determined using Fisher's protected least significant differences post hoc tests. Group differences for interactions were determined using one-way ANOVAs with alpha level corrected for multiple comparisons. Simple main effects and/or simple contrasts are presented following significant interactions. Results of post hoc comparisons are presented in parentheses. Alpha level for statistical significance was $p = 0.05$. Proestrous and OVX control group data are provided for reference (Table 2).

Results

Neuroendocrine status is influenced by adrenalectomy and FGIN 1-27

Central manipulations influenced neuroendocrine status. Compared to vehicle, subsequent infusions of FGIN 1-27 to the midbrain VTA significantly enhanced $3\alpha,5\alpha$ -THP concentrations in midbrain [$F(1,118)=20.85, p<0.05$], hippocampus [$F(1,118)=22.73, p<0.05$], cortex [$F(1,118)=22.24, p<0.05$], and diencephalon [$F(1,118)=20.56, p<0.05$], but not plasma (Table 3). Neither MK-801, nor bicuculline, significantly altered steroid concentrations. Concentrations of steroids measured in the brain were not significantly different between partially vs. completely ADX rats (Table 3).

Peripheral manipulations also influenced neuroendocrine status. OVX rats were assigned to partially or completely ADX groups via observed concentrations of circulating corticosterone (corticosterone $>1.0 \mu\text{g/dl}$ indicated incomplete ADX). As such, corticosterone was greater among rats that were considered partially ADX compared to those that were considered completely ADX [$F(1,118)=62.75, p<0.05$] (Table 3). As well, partially ADX rats had significantly greater P [$F(1,118)=4.91, p<0.05$] and E [$F(1,118)=3.93, p<0.05$] concentrations in plasma compared to completely ADX rats (Table 3).

Exploration is influenced by adrenalectomy and interaction of central manipulations

While infusions of GBR or NMDAR blockers did not appear to alter motor behavior, there was a main effect for subsequent infusions of FGIN 1-27 to significantly reduce the number of grid entries in the open field compared to subsequent infusions of vehicle [$F(1,118)=4.71, p<0.05$] (Table 4). No motor differences were observed in the elevated plus maze as determined by the number of arms entered (Table 4).

Central blocker and FGIN 1-27 infusions significantly interacted to influence time spent on the open arms of the elevated plus maze [$F(2,118)=2.89, p=0.05$] (Fig. 2). Simple main effects of the blocker condition revealed that, among rats receiving subsequent infusions of vehicle, MK-801 significantly increased the open arm time compared to infusions of vehicle ($p=0.01$) or bicuculline ($p=0.01$) which did not differ [$F(2,66)=4.47, p=0.02$] (see the single caret symbol in Fig. 2). However, among rats that received subsequent infusions of FGIN 1-27, open arm time did not significantly differ with blocker administration. Simple contrasts did not reveal significant differences for subsequent infusions of FGIN 1-27 to alter responding to blocker infusions compared to subsequent infusions of vehicle. No interaction with ADX condition was observed among central manipulations. No differences were observed in exploration of a novel conspecific (Table 1). Peripheral manipulations also influenced exploration. There was a main effect for open arm time to be lower with complete, versus partial, ADX [$F(1,118)=4.99, p<0.05$] (see the single asterisk in Fig. 2).

Paced mating is influenced by adrenalectomy and interaction of central manipulations

Central manipulations influenced sexual behavior. Irrespective of ADX condition, a significant main effect was observed for intra-VTA infusions of receptor blockers to alter the proportion of rats that engaged in mating [$F(2,118)=5.11, p<0.05$] (Fig. 3, top). Post hoc analyses revealed that infusions of MK-801 significantly increased engagement in mating compared to infusions of vehicle ($p=0.02$) or bicuculline ($p=0.0004$), which did not differ (see the double asterisks in Fig. 3, top).

Infusions of MK-801, bicuculline, and/or FGIN 1-27 interacted to influence lordosis quotients. Independent of ADX, there was a significant interaction between central blocker condition and central FGIN 1-27 conditions [$F(2,118)=5.72, p<0.05$] (Fig. 3, bottom). Simple main effects of the blocker condition revealed that, among rats receiving subsequent infusions of vehicle, MK-801 significantly enhanced lordosis quotients compared to infusions of vehicle ($p<0.0001$) or bicuculline ($p<0.0001$), which did not differ [$F(2,66)=14.78, p<0.0001$] (see the single caret symbol in Fig. 3, bottom). However, among rats that received subsequent infusions of FGIN 1-27, lordosis quotients did not significantly differ with blocker administration. Simple contrasts revealed that, compared to subsequent infusions of vehicle, subsequent infusions of FGIN 1-27 significantly enhanced lordosis quotients when administered following vehicle ($p=0.02$) and significantly reduced lordosis quotients when administered following MK-801 ($p=0.03$), whereas no differences were observed when administered following bicuculline (see the single number sign in Fig. 3, bottom).

Peripheral manipulations also influenced sexual behavior. There were main effects of ADX condition wherein a lower proportion of completely ADX rats engaged in mating [$F(1,118)=5.30, p<0.05$] (see the single asterisk in Fig. 3, top), responded to mounting with lordosis [$F(1,118)=5.76, p<0.05$] (see the single asterisk in Fig. 3, bottom), displayed defensive aggression [$F(1,118)=10.80, p<0.05$], and paced their mating contacts [$F(1,118)=6.77, p<0.05$] than did partially ADX rats (Table 1). No interaction with ADX condition was observed.

Discussion

The findings of the present investigation supported our hypotheses that complete ADX would reduce exploration and engagement in mating compared to partial ADX and that MK-801 or FGIN 1-27 would enhance these behaviors. The present investigation extends our understanding of how neurosteroids may influence NMDAR- and GBR-mediated behavior. Indeed, rats with partial ADX demonstrated greater baseline levels of engagement in mating, lordosis, pacing of sexual contacts, time exploring the open arms of an elevated plus maze, and greater circulatory concentrations of corticosterone, E, and P, compared to rats with complete ADX; however, ADX condition did not interact with any intra-VTA manipulation. Infusions of FGIN 1-27 decreased motor behavior in an open field compared to infusions of vehicle, without altering the number of arms that rats explored in an elevated plus maze. These data may suggest that spontaneous, but not goal-directed, motor behavior was reduced by the present regimen. Infusions of the NMDAR blocker, MK-801, enhanced exploration time on the elevated plus maze and the percentage of rats that engaged in mating; whereas, MK-801 or the neurosteroidogenesis enhancer, FGIN 1-27, enhanced the frequency of lordosis in response to a sexual contact. Of interest, FGIN 1-27 attenuated the lordosis-enhancing effects of MK-801 when co-administered. As such, NMDARs may be important substrates in these effects. The influence of GBRs may also be inherent. Although, bicuculline did not significantly reduce the already low levels of exploration or lordosis that were observed among OVX/ADX rats, bicuculline did block the FGIN 1-27-induced enhancement of lordosis among female rats. We have previously observed

bicuculline to modestly reduce open arm time and lordosis among intact, or OVX E-primed, rats (Table 2), suggesting that the complete ovary and adrenal extirpation, utilized herein, yielded a floor effect. These data suggest that NMDARs and/or GBRs in the VTA may be targets of neurosteroids effects to influence exploration and engagement in mating.

Neurosteroids may have reinforcing properties via actions in the VTA. Foremost, neurosteroids are rewarding as they readily condition a place preference (Finn et al. 1997; Rosellini et al. 2001) and are systemically self-administered by rodents preferentially over vehicle (Frye et al. 2007a; Sinnott et al. 2002). The paced mating paradigm allows the female the opportunity to “self-administer” her copulatory contacts as occurs in the natural environment (Calhoun 1962). In rodent mating, the female must flee and return to the male in an approach/avoidance manner in order to receive more intensive stimulation that will lead to conception. Despite the fact that all females in the present study received mounts, and did not significantly differ in their lordosis response to mounting irrespective of central/peripheral manipulations (Table 1), those that were partially ADX and/or infused with MK-801 did escalate mating to the point of intromission as depicted in Fig. 3 (top). Neurosteroid actions in the VTA may partly underlie the reinforcing aspects of mating that promote return to the male for more intensive stimuli. This is consistent with the notion that the midbrain acts as a relay module to coordinate endocrine and motoric activity to, and from, hypothalamus and hindbrain sites (Pfaff et al. 2008). Intra-VTA actions at NMDARs and GBRs may be important for these effects.

Neurosteroids, including $3\alpha,5\alpha$ -THP, are allosteric modulators that can act at neurotransmitter targets (Callachan et al. 1987). In particular, pregnane neurosteroids can increase the potency of glutamate and glycine on their cognate receptors (Johansson and Le Grevès 2005; Malayev et al. 2002). We have previously demonstrated that intra-VTA blockade of NMDARs can enhance rodent engagement in lordosis (Frye et al. 2008a; Petralia et al. 2007). The current findings confirm and extend these reports to demonstrate enhanced motivation to engage in copulation, as indicated by increased pacing of sexual contacts among partially ADX rats (commensurate with observations in adrenally intact rats in Table 2) and an increased proportion of MK-801-infused rats engaging in mating compared to other groups. Co-administration of MK-801 with FGIN 1-27 attenuated enhancement of lordosis. One explanation may be that FGIN 1-27 acts synergistically with MK-801 (via enhancement of $3\alpha,5\alpha$ -THP) to push the dose-response in a U-shaped curve. In support, MK-801's depression-modulating effects demonstrate an inverted U-shaped dose-response curve in mice (Dhir and Kulkarni 2008), as do $3\alpha,5\alpha$ -THP's effects on social aggressive behavior when co-administered with a GABAergic modulator, such as alcohol (Fish et al. 2001). $3\alpha,5\alpha$ -THP's biphasic effects on aggression have been noted. Among women in withdrawal from P-based hormone therapy, negative mood is increased (Bjorn et al. 2002) and a greater reduction of circulatory $3\alpha,5\alpha$ -THP is observed when women experience withdrawal from therapies that include P vs. those comprised of E alone (Wihlbäck et al. 2007). Negative changes in mood may be partly related to upregulation of GBRs with a subunit composition that is insensitive to typical GBR agonists (Smith et al. 2007). In the present study, it is of interest that defensive aggression was greater among partially ADX rats compared to completely ADX rats. Protracted withdrawal from endogenous steroids may be greater when peripheral sources are perturbed rather than extirpated.

Investigations aiming to elucidate dynamic relationships that may exist between social interactions and pharmacological/neurobiological response have been understudied (Cacioppo et al. 2007). The present study suggests a role for neurosteroids to have effects to facilitate and maintain lordosis which may occur via reinforcing actions at intra-VTA NMDARs and/or GBRs that maintain copulation. However, the present investigation had

some limitations. Adrenal regeneration may be presumed by the corticosterone response observed in some rats that were considered partially ADX, but could not be directly assessed. As such, individual differences in the magnitude of adrenal regeneration among partially ADX rats are not known. Future investigations should assess diurnal rhythm in order to compare rats at the time of daily corticosterone peak. Together, these data suggest that actions of neuroactive steroids in the VTA may be important targets for future investigations of social and hedonic behavior.

Acknowledgments

This work was supported by funding from the National Institute of Mental Health (MH06769801). Technical assistance provided by Danielle Llaneza, Daniel Cusher, and Eric Zhou is appreciated.

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Fig. 1.
An example of fresh tissue verification of site placement

Fig. 2.

The effects of vehicle, MK-801 (200 ng), or bicuculline (100 ng) to the midbrain VTA followed by subsequent infusions of vehicle or FGIN 1-27 (5 μ g) on open arm time (mean $\pm$ SEM) in the elevated plus maze. *Single asterisk* denotes performance of partially adrenalectomized rats is significantly different from that of completely adrenalectomized rats. *Single caret* denotes performance of MK-801/vehicle-infused rats is significantly different from vehicle/vehicle-infused rats ($p < 0.05$)

Fig. 3.

The effects of vehicle, MK-801 (200 ng), or bicuculline (100 ng) to the midbrain VTA followed by subsequent infusions of vehicle or FGIN 1-27 (5 μ g) on the percentage of rats that engage in mating (*top*) and their frequency of lordosis (*bottom*; mean±SEM). *Single asterisk* denotes performance of partially adrenalectomized rats is significantly different from that of completely adrenalectomized rats. *Double asterisks* denote performance of MK-801-infused rats is significantly different from that of vehicle- or bicuculline-infused rats. *Single caret* denotes performance of MK-801/vehicle-infused rats is significantly different from vehicle/vehicle-infused rats. *Single number sign* denotes performance of rats, subsequently infused with FGIN 1-27, is significantly different than that of rats subsequently infused with vehicle ($p<0.05$)

Depicts frequency of aggression and pacing following mating contacts in the paced mating task (mean±SEM), as well as time spent exploring a novel conspecific in the social interaction task (secs±SEM) among ovariectomized, estradiol-primed rats that were partially or completely adrenalectomized (mean±SEM)

Table 1

Intra-VTA Infusate #1	Partial adrenalectomy				Complete adrenalectomy					
	Vehicle		MK-801		Bicuculline			Vehicle		
	Vehicle (n=10)	FGIN (n=10)	Vehicle (n=12)	FGIN (n=14)	Vehicle (n=9)	FGIN (n=10)	Vehicle (n=8)	Vehicle (n=11)	FGIN (n=12)	Vehicle (n=11)
Intra-VTA Infusate #2	Vehicle (n=10)	FGIN (n=10)	Vehicle (n=12)	FGIN (n=14)	Vehicle (n=9)	FGIN (n=10)	Vehicle (n=8)	Vehicle (n=11)	FGIN (n=12)	Vehicle (n=11)
Paced mating										
Responding to mounting with lordosis (%)	40±16	42±15	36±13	22±15	20±13	25±16	10±10	23±12	50±15	13±13
Aggression quotient	75±13	77±12	74±10	85±11	33±15	50±16	31±13 ^a	34±12 ^a	46±13 ^a	50±19 ^a
Pacing (%)	20±7	33±10	21±4	20±7	22±11	33±15	10±5 ^a	8±3 ^a	20±7 ^a	4±3 ^a
Social interaction										
Exploration time	94±15	81±12	98±14	86±20	62±16	73±12	94±10	84±12	97±13	90±14

Rats received infusions of vehicle, MK-801 (200 ng), or bicuculline (100 ng) to the midbrain VTA followed by subsequent infusions of vehicle or FGIN 1-27 (FGIN; 5 µg)

^aSignificant difference in the main effect of complete adrenalectomy from partial adrenalectomy

Table 2

Depicts behavioral responding (mean±SEM) of gonadally intact, proestrous rats or rats that have been ovariectomized and hormone-primed with estradiol (10 µg, SC, 44 h prior to testing) and progesterone (50 µg, SC, 3 h prior to testing) in the open field, elevated plus maze, social interaction, and paced mating tasks

Intra-VTA infusate	Gonadally/adrenally intact, proestrous			Ovariectomized, hormone-primed		
	Vehicle (n=5)	MK-801 (n=4)	Bicuculline (n=4)	Vehicle (n=3)	MK-801 (n=3)	Bicuculline (n=3)
Open field						
Total entries	305±25	333±17	227±21	128±13	225±14	177±10
Elevated plus maze						
Total arm entries	12±2	21±3	12±3	15±1	18±2	11±3
Open arm time (s)	33±5	84±14	43±23	16±3	24±4	2±2
Social interaction						
Interaction time (s)	107±15	89±12	62±11	62±10	44±1	20±2
Paced mating						
Mating (%)	100±0	100±0	50±50	100±0	100±0	33±33
Lordosis quotient	98±2	94±6	47±27	83±17	98±2	28±28
Aggression quotient	18±8	6±6	14±14	22±22	0±0	47±24
Pacing (%)	14±5	23±5	6±5	50±25	25±14	7±7

All rats were infused with vehicle, MK-801 (200 ng), or bicuculline (100 ng) to the midbrain VTA 30 min prior to testing

Depicts estradiol, progesterone, dihydroprogesterone (DHP), 5 α -pregnan-3 α -ol-20-one (3 α ,5 α -THP), and/or corticosterone concentrations in midbrain, hippocampus, cortex, diencephalon, and plasma of ovariectomized, estradiol-primed rats that were partially or completely adrenalectomized (mean $\pm$ SEM)

Table 3

		Partial adrenalectomy				Complete adrenalectomy							
Intra-VTA Infusate #1	Intra-VTA Infusate #2	Vehicle		MK-801		Bicuculline		Vehicle		MK-801		Bicuculline	
		Vehicle (n=10)	FGIN (n=12)	Vehicle (n=14)	FGIN (n=9)	Vehicle (n=10)	FGIN (n=8)	Vehicle (n=11)	FGIN (n=13)	Vehicle (n=12)	FGIN (n=8)	Vehicle (n=12)	FGIN (n=11)
Midbrain													
Estradiol (pg/g)	1.9±0.3	1.8±0.3	2.2±0.6	1.6±0.2	2.2±0.4	2.0±0.2	1.9±0.3	1.7±0.4	1.9±0.3	1.6±0.2	1.6±0.1	1.8±0.2	1.8±0.2
Progesterone (ng/g)	0.9±0.2	1.0±0.1	0.9±0.1	1.1±0.1	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1	1.1±0.1	1.1±0.1	1.0±0.1	1.1±0.1	1.1±0.1
DHP (ng/g)	6.8±1.5	6.8±0.6	6.9±0.8	7.9±0.5	7.2±0.9	8.3±0.5	7.5±0.8	7.0±0.7	8.0±0.6	8.3±0.8	7.0±0.9	7.8±0.3	7.8±0.3
3α,5α-THP (ng/g)	1.3±0.5	2.4±0.6 ^b	1.4±0.6	2.8±0.8 ^b	0.5±0.2	2.5±0.6 ^b	1.6±0.8	2.9±0.6 ^b	0.3±0.1	2.2±0.7 ^b	1.1±0.4	2.2±0.6 ^b	2.2±0.6 ^b
Hippocampus													
Estradiol (pg/g)	0.9±0.1	0.7±0.1	0.8±0.1	0.6±<0.0	0.9±0.1	0.9±0.2	0.8±0.1	0.6±0.1	0.6±0.1	0.7±0.1	0.6±0.1	0.8±0.1	0.8±0.1
Progesterone (ng/g)	0.4±<0.0	0.4±<0.0	0.4±<0.0	0.5±<0.0	0.4±<0.0	0.4±<0.0	0.3±0.1	0.4±<0.0	0.4±<0.0	0.4±<0.0	0.4±<0.0	0.4±<0.0	0.4±<0.0
DHP (ng/g)	2.7±0.5	2.8±0.2	2.7±0.2	3.3±0.2	2.5±0.1	2.8±0.2	2.8±0.3	3.1±0.2	3.1±0.2	2.9±0.2	2.6±0.2	3.0±0.2	3.0±0.2
3α,5α-THP (ng/g)	1.1±0.3	2.7±0.6 ^b	0.5±0.2	2.8±0.9 ^b	0.3±0.2	1.8±0.8 ^b	1.4±0.4	2.7±0.9 ^b	1.2±0.6	3.9±1.1 ^b	1.1±0.4	2.4±0.8 ^b	2.4±0.8 ^b
Cortex													
Estradiol (pg/g)	1.1±0.2	0.9±0.1	1.2±0.2	0.9±0.1	1.2±0.2	1.0±0.2	1.1±0.2	0.8±0.1	0.9±0.1	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1
Progesterone (ng/g)	0.5±0.1	0.6±<0.0	0.5±<0.0	0.5±<0.0	0.5±<0.0	0.5±0.1	0.5±0.1	0.5±0.1	0.5±<0.0	0.5±0.1	0.6±<0.0	0.5±<0.0	0.5±<0.0
DHP (ng/g)	3.2±0.5	3.9±0.3	3.4±0.3	3.5±0.2	3.8±0.3	3.7±0.2	3.4±0.3	4.1±0.4	3.9±0.2	4.2±0.3	3.9±0.3	3.9±0.2	3.9±0.2
3α,5α-THP (ng/g)	1.8±0.5	3.4±0.9 ^b	1.0±0.4	2.8±1.0 ^b	0.7±0.2	2.4±0.8 ^b	1.9±0.7	3.6±0.9 ^b	0.5±0.2	2.8±1.2 ^b	1.0±0.5	3.6±0.8 ^b	3.6±0.8 ^b
Diencephalon													
Estradiol (pg/g)	2.7±0.3	2.8±0.3	3.4±0.6	2.3±0.3	3.2±0.3	2.9±0.4	2.9±0.2	2.3±0.2	2.9±0.2	3.3±0.5	2.7±0.3	3.2±0.4	3.2±0.4
Progesterone (ng/g)	1.4±0.2	1.7±0.1	1.7±0.1	1.6±0.1	1.7±0.2	1.7±0.2	1.6±0.2	1.7±0.2	1.8±0.2	1.7±0.2	1.7±0.1	2.0±0.2	2.0±0.2
DHP (ng/g)	0.9±0.2	1.2±0.1	1.2±0.1	1.2±0.1	1.2±0.2	1.1±0.2	1.2±0.1	1.2±0.1	1.3±0.1	1.3±0.2	1.1±0.1	1.2±0.2	1.2±0.2
3α,5α-THP (ng/g)	1.1±0.3	2.7±0.6 ^b	0.5±0.2	2.8±0.9 ^b	0.3±0.2	1.8±0.8 ^b	1.4±0.4	2.7±0.9 ^b	1.2±0.6	3.9±1.1 ^b	1.1±0.4	2.4±0.8 ^b	2.4±0.8 ^b
PLASMA													
	2.1±0.4	2.1±0.6	1.7±0.3	2.2±0.4	2.9±0.9	2.2±0.9	0.3±0.1 ^a	0.3±0.1 ^a	0.3±0.1 ^a	0.4±0.1 ^a	0.2±0.1 ^a	0.4±0.1 ^a	0.4±0.1 ^a
Corticosterone (µg/dl)	27.0±1.7	21.5±2.4	24.6±1.2	24.1±1.2	25.5±1.2	26.1±2.4	21.1±1.0 ^a	21.6±2.6 ^a	21.6±1.1 ^a	24.2±1.3 ^a	25.4±1.9 ^a	22.0±2.1 ^a	22.0±2.1 ^a
Estradiol (pg/ml)	0.7±0.1	0.6±0.1	0.6±0.1	0.6±0.1	0.6±0.1	0.5±0.1	0.5±0.1 ^a	0.5±0.1 ^a	0.5±0.1 ^a	0.5±0.1 ^a	0.6±0.1 ^a	0.4±0.1 ^a	0.4±0.1 ^a
Progesterone (ng/ml)	1.1±0.2	1.1±0.2	1.1±0.2	1.2±0.3	1.3±0.2	1.5±0.4	1.1±0.2	0.6±0.2	0.6±0.2	1.2±0.4	1.3±0.2	1.0±0.3	1.0±0.3

Partial adrenalectomy		Complete adrenalectomy							
Intra-VTA Infusate #1	Vehicle		MK-801				Bicuculline		
	Vehicle	FGIN (n=10)	FGIN (n=12)	Vehicle (n=12)	Vehicle (n=14)	FGIN (n=9)	Vehicle (n=9)	Vehicle (n=10)	FGIN (n=8)
Intra-VTA Infusate #2	Vehicle (n=10)	FGIN (n=10)	FGIN (n=12)	Vehicle (n=12)	Vehicle (n=14)	FGIN (n=9)	Vehicle (n=9)	Vehicle (n=10)	FGIN (n=8)
DHP (ng/ml)	2.5±0.5	2.1±0.6	2.1±0.6	1.8±0.5	1.7±0.3	2.2±0.4	1.7±0.7	1.4±0.5	1.5±0.6
3α,5α-THP (ng/ml)	2.1±0.4	2.1±0.6	2.1±0.6	1.7±0.3	1.7±0.3	2.2±0.4	2.2±0.4	2.9±0.9	2.2±0.9
				0.3±0.1 ^a	0.3±0.1 ^a	0.3±0.1 ^a	0.3±0.1 ^a	0.3±0.1 ^a	0.3±0.1 ^a
				1.7±0.6	1.7±0.6	1.7±0.6	1.7±0.6	1.7±0.6	1.7±0.6
				1.7±0.5	1.7±0.5	1.7±0.5	1.7±0.5	1.7±0.5	1.7±0.5
				0.4±0.1 ^a	0.4±0.1 ^a	0.4±0.1 ^a	0.4±0.1 ^a	0.4±0.1 ^a	0.4±0.1 ^a
				1.2±0.3	1.2±0.3	1.2±0.3	1.2±0.3	1.2±0.3	1.2±0.3
				1.8±0.5	1.8±0.5	1.8±0.5	1.8±0.5	1.8±0.5	1.8±0.5
				0.2±0.1 ^a	0.2±0.1 ^a	0.2±0.1 ^a	0.2±0.1 ^a	0.2±0.1 ^a	0.2±0.1 ^a

Rats received infusions of vehicle, MK-801 (200 ng), or bicuculline (100 ng) to the midbrain VTA followed by subsequent infusions of vehicle or FGIN 1-27 (FGIN; 5 µg)

^aSignificant difference in the main effect of complete adrenalectomy from partial adrenalectomy ($p<0.05$)

^bSignificant difference in the main effect of infusions of FGIN 1-27 from infusions of vehicle ($p<0.05$)