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3α-HYDROXY-5α-PREGNAN-20-ONE IN THE MIDBRAIN VENTRAL TEGMENTAL AREA MEDIATES SOCIAL, SEXUAL, AND AFFECTIVE BEHAVIORS

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Abstract

Progestins mediate the onset and duration of lordosis, the mating posture of female rodents, through actions in the hypothalamus and ventral tegmental area. In the hypothalamus, progesterone has traditional, "genomic" actions via intracellular progestin receptors. In the ventral tegmental area, 3α-hydroxy-5α-pregnan-20-one has "non-genomic" actions independent of progestin receptors to facilitate lordosis that involve $GABA_A$/benzodiazepine receptors, NMDA type glutamate receptors, and/or dopamine receptors. 3α-Hydroxy-5α-pregnan-20-one levels also change with behavioral and/or environmental stimuli and may have a role in other reproductively-relevant behaviors, such as affiliation, exploration, and anxiety (socio-sexual behaviors). Data are reviewed that support the notion that: 1) effects of 3α-hydroxy-5α-pregnan-20-one in the midbrain ventral tegmental area facilitate lordosis and other reproductively-relevant behaviors. 2) 3α-Hydroxy-5α-pregnan-20-one, formed in the ventral tegmental area from metabolism of progestins, produced peripherally by endocrine glands, or centrally from biosynthesis in glial cells mediates socio-sexual behaviors. 3) 3α-Hydroxy-5α-pregnan-20-one's actions at $GABA_A$/benzodiazepine receptors, NMDA type glutamate receptors, and dopamine receptors in the ventral tegmental area are important for lordosis; however, effects at these substrates on socio-sexual behaviors have not been elucidated. Given 3α-hydroxy-5α-pregnan-20-one's involvement in stress responses, its putative role as a homeostatic regulator and in the pathophysiology and treatment of neuropsychiatric disorders is discussed.

Keywords

steroidogenesis; $GABA_A$ receptors; NMDA receptors; dopamine; stress response; mood

Our research focuses on the mechanisms of progestins' actions in the midbrain ventral tegmental area (VTA) that mediate the onset and duration of sexual behavior of female rodents. To date, the majority of our investigations have utilized a model system in which progestins' mechanisms are manipulated in the VTA and/or the hypothalamus of ovariectomized (OVX), estrogen (E)-primed rodents. The effects on lordosis, the stereotypic posture which female

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rodents assume to enable mating to occur (given appropriate hormonal and environmental stimulation), are then used as a bioassay to indicate which mechanisms of progestins are required for lordosis. This approach has revealed that progesterone (P) has classic actions through intracellular progestin receptors (PRs-"genomic" actions) in the hypothalamus to initiate lordosis, but P's metabolite, 3*α*-hydroxy-5*α*-pregnan-20-one (3*α*,5*α*-THP), has "non-genomic" actions independent of PRs in the VTA to mediate the incidence and quality of lordosis. The present focus of our laboratory is to ascertain how the effects and mechanisms of 3*α*,5*α*-THP in the VTA for lordosis extend to other aspects of mating. This paper summarizes our research on 3*α*,5*α*-THP's effects and mechanisms in the VTA for lordosis, describes results of our initial investigations on the role of 3*α*,5*α*-THP in the VTA for other social and affective behaviors, and discusses the relevance of 3*α*,5*α*-THP and these investigations for neuropsychiatric disorders.

3α,5α-THP's effects and mechanisms in the VTA for lordosis

The importance of the VTA for lordosis

Localization, hormone implant, and lesion studies reveal that P's actions in the VTA are important for mediating lordosis of E-primed rodents. Accumulation of ^{3}H P is greater in the midbrain than the hypothalamus (Whalen and Luttge, 1971). P alone to the VTA enhances lordosis or augments lordosis facilitation initiated by P implants to the hypothalamus (Pleim et al., 1991). Lesions to the VTA, or cutting connections between the VTA and hypothalamus, disrupts initiation and maintenance, respectively, of P-facilitated lordosis (Rose, 1990). Thus, the VTA is important for progestins' actions to facilitate sexual behavior.

Metabolism to, and biosynthesis of, 3α,5α-THP in the VTA is important for mediating lordosis

P's effects on lordosis are mediated, in part, by 3*α*,5*α*-THP in the VTA. Levels of P and 3*α*, 5*α*-THP are increased coincident with lordosis. Circulating and midbrain levels of P and 3*α*, 5*α*-THP rise on proestrus, high levels are sustained for 10-12 h, when mating typically occurs, and progestin levels decrease coincident with estrous termination (Frye and Bayon, 1999).

In the VTA, P's metabolism to 3*α*,5*α*-THP is critical for lordosis. Progestins secreted by the ovaries and/or adrenals are metabolized centrally by actions of 5*α*-reductase (5*α*-R) and 3-hydroxysteroid oxidoreductase enzymes (3-HSOR; Zanisi et al., 1984). These enzymes have been identified in the VTA by *in situ* hybridization and/or immunocytochemistry (Frye, 2001). Manipulating these enzymes in the VTA alters lordosis and midbrain 3*α*,5*α*-THP levels. Inhibiting P's metabolism to 3*α*,5*α*-THP attenuates lordosis of rodents. Systemic or intra-VTA 5*α*-R or 3-HSOR inhibitors similarly decrease lordosis and 3*α*,5*α*-THP levels of naturally-receptive or hormone-primed rodents (Frye, 2001; Frye and Vongher, 2001; Petralia et al., in preparation). Mice deficient in 5*α*-R, when administered P, do not demonstrate enhanced social and/or affective behaviors as do their wild-type counterparts (Frye et al., 2004a). Enhancing activity of these metabolism enzymes in the VTA facilitates lordosis and formation of 3*α*,5*α*-THP (Mellon and Griffin, 2002; Frye and Seliga, 2003). These data suggest that metabolism of P to 3*α*,5*α*-THP in the VTA is essential for facilitation of lordosis.

3*α*,5*α*-THP, formed in part from central biosynthesis, may also be important for lordosis. Mitochondrial benzodiazepine receptors (MBRs) in glial cells facilitate cholesterol transport from the outer to the inner mitochondrial membrane. The P450 side chain cleavage enzyme (P450scc) then converts cholesterol to pregnenolone, which is then metabolized by 3*β*-hydroxysteroid dehydrogenase (3*β*-HSD) to P. The necessary enzymes (P450scc, 3*β*-HSD) for biosynthesis of 3*α*,5*α*-THP have been identified in the midbrain VTA (Mellon and Griffin, 2002). Infusions to the VTA of MBR antagonists or agonists, respectively, decrease and increase lordosis and midbrain 3*α*,5*α*-THP levels of naturally-receptive or hormone-primed

rodents (Frye and Petralia, 2003a,b). Implants of a P450scc inhibitor or a 3*β*-HSD inhibitor reduce lordosis of naturally-receptive or hormone-primed rodents to levels similar to that seen following P metabolism inhibition (Frye and Vongher, 2001; Petralia et al., in preparation). Co-administration of biosynthesis and metabolism inhibitors do not further reduce lordosis below that produced by biosynthesis or metabolism inhibitors alone (Petralia et al., in preparation). Thus, biosynthesis of neurosteroids in the VTA may be essential for 3*α*,5*α*-THP-facilitated lordosis.

Although these data indicate that formation of 3*α*,5*α*-THP in the midbrain VTA is critical for lordosis, they do not directly address the substrates through which 3*α*,5*α*-THP may have its actions in the VTA to facilitate lordosis. This is discussed below.

Progestins' effects in the VTA for lordosis are independent of PRs and membrane-mediated

Progestins actions in the VTA that mediate lordosis are independent of PRs. Receptor binding, autoradiography, and immunocytochemical studies reveal only few PRs in the VTA that are not E-induced, despite the requirement of E-priming for P's action at PRs (Frye, 2001; Blaustein, 2003). Blocking P's actions at PRs in the VTA with antagonists or anti-sense oligonucleotides does not attenuate lordosis (Frye and Vongher, 1999a). Furthermore, progestins' affinity for PRs does not predict their lordosis-enhancing effects when applied to the VTA (Frye and Vongher, 1999b). 3*α*,5*α*-THP is devoid of affinity for PRs (Iswari et al., 1986), but is the most effective progestin at facilitating lordosis when applied to the VTA (Frye and Vongher, 1999b). Thus, progestins' actions in the VTA for lordosis are independent of PRs.

Progestins' actions in the VTA for lordosis are membrane-mediated. First, the latency of progestins to facilitate lordosis can be very rapid. Within 1-5 min of i.v. or intra-VTA infusion of progestins, there are increases in neuronal firing and lordosis (Rose, 1990; Frye et al., 2000a). Rapid effects of progestins are unlikely due to formation of new proteins produced by progestins' interaction with intracellular PRs, as this minimally requires 10-15 min (Pfaff and McEwen, 1983). Second, membrane-limited effects of progestins in the VTA are sufficient to rapidly facilitate lordosis. P bound to macromolecules, are too large and hydrophobic to permeate, but can bind to and have effects on, neuronal membranes (Ke and Ramirez, 1990). P conjugates, or free P, similarly facilitate receptivity within minutes of application to the VTA (Rose, 1990; Frye and DeBold, 1992; Frye and Gardiner, 1996). Similar effects of free and conjugated P to increase neuronal excitability in the VTA of PR knockout and wild-type mice, suggest that progestin conjugates do not produce their behavioral effects by becoming unbound and acting at PRs (Frye and Vongher, 1999c). Thus, the rapid effects of free and membrane-bound progestins indicate that membrane constituents are likely targets of progestins' actions in the VTA to facilitate lordosis.

3α,5α-THP acts in the VTA via GBRs

Anatomical and biochemical studies indicate that progestins modify $GABA_A$/benzodiazepine receptors (GBRs). GBRs are membrane-bound proteins with interacting binding sites (barbiturates, benzodiazepine, GABA), coupled to a chloride ionophore channel, which mediate the inhibitory neurotransmitter GABA. Progestins are positive, allosteric modulators of GBRs, which increase binding of GABA to GBRs. Number, density, and affinity of GBRs are enhanced when progestin levels are elevated to those that would facilitate receptivity in rodents (Wilson, 1996).

Progestins vary in their ability to enhance GBR function. Structure activity relationships indicate that 3*α*,5*α*-THP, with A-ring reduction at the five carbon, is the most potent, positive, endogenous modulator of GBRs (Majewska et al., 1986). 3*α*,5*α*-THP is more effective than P

at enhancing GBR function. 3*α*,5*α*-THP more rapidly inhibits binding of the convulsant tertbutylbicyclophosphorothionate to the GABA-operated chloride channel, potentiates GABA's effects on chloride uptake, and increases flunitrazepam binding more than does P (Majewska et al., 1986). 3*α*,5*α*-THP is about 600× more potent than the most effective barbiturates, and 60× more potent than P itself, such that, in nanomolar concentrations, 3*α*,5*α*-THP increases binding of endogenous GABA to GBRs (Majewska et al., 1986). Thus, GBRs in the VTA are putative substrates for 3*α*,5*α*-THP's actions.

Robust changes in midbrain 3*α*,5*α*-THP over the estrous cycle and with mating occur concomitant with endogenous variations in GABA and GBR sensitivity (Frye, 2001). Midbrain GABA levels are greater during behavioral estrus, than diestrus, and are highest after mating (Frye, 2001). As well, during behavioral estrus, less GABA is needed to displace muscimol, a GBR agonist, from its binding sites in midbrain tissues (Frye, 2001). These findings suggest that coincident with sexual receptivity, more GABA and greater GBR function can occur in the midbrain VTA.

Manipulating GABA and/or GBR function in the VTA alters progestin-facilitated lordosis. Intra-VTA infusions of anti-sense oligonucleotides for glutamic acid decarboxylase (GAD), which synthesizes GABA from glutamate, disrupt lordosis and reduce GAD expression compared with vehicle or sense infusions (Frye and Vongher, 1999a; Frye et al., 2000b). Preventing GABA degradation by infusions of a GABA-transaminase inhibitor to the VTA facilitates lordosis (Frye et al., 1993). Infusions of GBR agonists and antagonists to the VTA, respectively, increase and decrease P-mediated lordosis of rodents (Frye et al., 1993; Frye, 2001). Similarly, progestins' rapid facilitation of lordosis when applied to the VTA corresponds with their ability to increase GBR activity in the VTA (Frye and Vongher, 1999b). Thus, altering GABA levels or GBR function in the VTA mediates progestin-facilitated lordosis of rodents.

Progestin-NMDA type glutamate receptor (NMDAR) interactions

NMDARs may also be substrates for progestins' actions. Progestins bind to, and alter the function of, NMDARs. Systemic, selective non-competitive antagonists at NMDARs, attenuate lordosis and motor behavior of OVX, hormone-primed rodents (Fleischmann et al., 1991; DeBold et al., 2000). Systemic P decreases glutamate excitation of Purkinje neurons (Smith et al., 1987).

3*α*,5*α*-THP's effects on lordosis may involve actions at NMDARs in the VTA. NMDARs are co-localized with GBRs in the VTA (Willick and Kokkinidis, 1995). Glutamate levels are increased in the midbrain of mated and receptive rodents that have elevated midbrain 3*α*,5*α*-THP compared with non-receptive rodents (Frye, 2001). Blocking P's metabolism to 3*α*,5*α*-THP reduces glutamate's excitatory effects (Park-Chung et al., 1997). Blocking NMDARs in the VTA enhances 3*α*,5*α*-THP-facilitated lordosis of rodents (DeBold et al., 2000; Frye and Petralia, 2003c). Thus, 3*α*,5*α*-THP-facilitated lordosis may involve glutamate and/or NMDAR function in the midbrain VTA.

Progestin-dopamine receptor interactions

Dopamine type 1 receptors (D_1) may be substrates for progestins' actions for lordosis. D_1 density is higher concomitant with elevated progestin levels during behavioral estrus than during diestrus (Levesque and Di Paolo, 1990). 3*α*,5*α*-THP and dopamine levels are greater in the midbrain of receptive compared with non-receptive rats, and are further increased with mating (Frye, 2001). I.V. or intra-VTA infusions of a D_1 antagonist attenuate, and of a D_1 agonist, enhance lordosis of naturally-receptive or hormone-primed rodents (Frye and

Vongher, 1999a; Frye et al., 2000a, 2004b; Petralia and Frye, 2004). Thus, in the VTA, progestins may facilitate lordosis, in part, via actions at D_1.

In the VTA, D_1 may be one of many substrates that mediate 3*α*,5*α*-THP's actions for lordosis. One question is how 3*α*,5*α*-THP's actions at D_1 and other substrates are related. Progestins' effects for lordosis through D_1 and GBRs may involve similar intracellular signaling pathways. Activation of D_1 or GBRs can lead to initiation of intracellular signaling cascades involving G-proteins and cyclic AMP (cAMP; Kalivas, 1993; Fancsik et al., 2000). Midbrain cAMP levels are higher in naturally-receptive than nonreceptive rats (Frye and Petralia, 2003c). Moreover, infusions to the VTA of G-proteins or cAMP inhibitors decrease, and cAMP analogues, increase 3*α*,5*α*THP-facilitated lordosis (Petralia and Frye, 2004). Given that both D_1 and GBRs can be metabotropic, inhibiting G-proteins and/or cAMP could block progestins' actions at either, both, and/or other substrates. Indeed, blocking common intracellular signaling pathways may explain why antagonizing GBRs, in the VTA, blocks D_1-mediated increases in 3*α*,5*α*-THP-facilitated lordosis (Frye, unpublished observations). Alternatively, the apposition of GBRs and D_1 in the VTA may explain such effects. Neuroanatomical studies reveal GABAergic terminals, which contain D_1, synapse on dopaminergic cell bodies that contain GBRs (Bayer and Pickel, 1991). Increasing activity of D_1 or GBRs leads to release of the other receptors' neurotransmitter. In the VTA, infusions of D_1 agonists increase GABA release and infusions of GBR agonists elevate somatodendritic dopamine release (Kalivas, 1993). Studies are ongoing to clarify whether progestins' actions in the VTA for lordosis via GBRs are up- or downstream of D_1.

The data discussed above indicate effects, sources, and substrates of 3*α*,5*α*-THP-facilitated lordosis. Briefly: (1) 3*α*,5*α*-THP in the VTA facilitates lordosis of rodents. (2) Central biosynthesis and/or metabolism of 3*α*,5*α*-THP in the VTA is important for lordosis. (3) GBRs, NMDARs, and/or D_1 in the VTA may be substrates through which 3*α*,5*α*-THP facilitates lordosis of rodents. Although we have ascertained some effects and mechanisms of 3*α*,5*α*-THP in the VTA for lordosis, how rapid changes in neurosteroids, such as 3*α*,5*α*-THP, produce local effects via multiple "non-genomic" substrates to alter neuronal activity and other processes, including behaviors relevant for mating, are also of great interest.

3α,5α-THP as an important homeostatic regulator

3*α*,5*α*-THP may be an important neuroendocrine factor that helps an organism respond to environmental challenges. 3*α*,5*α*-THP is present during prenatal development and increases in response to stressors, such as maternal separation, as early as postnatal day 6 (Kellogg and Frye, 1999; Kehoe et al., 2000; McCormick et al., 2002). In adults, increases in 3*α*,5*α*-THP in response to acute cold-water swim, shock, ether, and/or carbon dioxide exposure have been demonstrated in intact, gonadectomized, and/or adrenalectomized animals (Paul and Purdy, 1992). More subtle stimuli, such as social challenge and/or mating, also alters neurosteroidogenesis (Frye and Bayon, 1999; Frye, 2001; Miczek et al., 2003). Increases in 3*α*,5*α*-THP produced by such experiences are conserved across species, enhance GBR function, produce anxiolysis, and attenuate activation of sympathetic/hypothalamic-pituitary-adrenal (HPA) responses, which may help individuals return to a state of homeostasis following challenge (Paul and Purdy, 1992; Patchev and Almeida, 1996; Barbaccia et al., 2001; Frye 2001; Mensah-Nyagan et al., 2001). Blocking formation of 3*α*,5*α*-THP or its actions at GBRs prevents anti-anxiety behavior and stress-induced glucocorticoid secretion (Rhodes and Frye, 2001; Reddy, 2002). We have begun investigating the functional relevance of changes in 3*α*, 5*α*-THP secretion by examining effects on reproductive behaviors in our model system.

3*α*,5*α*-THP levels in the midbrain vary considerably with hormone-priming and/or mating stimuli. In order for rodents to be sexually receptive, ovarian secretion or exogenous

administration of E is necessary. E increases the formation of central progestins, the activity of the 5*α*-R enzyme, and formation of 3*α*,5*α*-THP (Cheng and Karavolas, 1973; Sinchak et al., 2003). Further increases in receptive behaviors are produced by ovarian secretion of progestins or exogenous administration of progestins, which increases midbrain levels of 3*α*,5*α*-THP over that of diestrous, OVX, or OVX, E-primed rodents (Frye and Bayon, 1999; Frye, 2001). Notably, following mating, midbrain 3*α*,5*α*-THP levels are increased over those of nonmated naturally-receptive or hormone-primed rodents (Frye, 2001). The rapidity of the mating-induced increase in midbrain 3*α*,5*α*-THP, and independence of secretion from the ovaries and/or adrenals, suggest that biosynthesis and subsequent metabolism of central, rather than peripheral, progestins underlie mating-induced increases in midbrain 3*α*,5*α*-THP that have been observed for rats, mice, and hamsters (Frye, 2001; Frye and Petralia, 2003a,b; Petralia et al., in preparation). The successive increases in midbrain 3*α*,5*α*-THP produced by E, P-priming, and mating have led us to consider whether 3*α*,5*α*-THP in varying concentrations, or when derived from peripheral versus central prohormones, may influence different aspects of reproductive behavior.

3*α*,5*α*-THP may help mediate individuals' adaptation to stressors that are encountered in social interactions. Mating requires that exploration is enhanced to allow for potential mates to be found. Fearful and/or anxious responses to potential mates must be suppressed so that approaches can be made toward stimuli that previously elicited aggressive responses (Carter et al., 1999). Interestingly, hormone regimens that enhance mating typically inhibit aggression, increase exploration, and produce anti-anxiety effects (DeBold and Miczek, 1981; Frye et al., 1998). Lower dosages of 3*α*,5*α*-THP, which would be expected to produce circulating concentrations akin to that of non-receptive rodents or those approaching estrous termination, heighten aggressive behavior, whereas higher dosages are anti-aggressive and enhance lordosis (Frye, 2001; Fish et al., 2002; Miczek et al., 2003; Petralia and Frye, 2004). Circulating 3*α*, 5*α*-THP levels are naturally elevated during behavioral estrus when rats are sexually-responsive and show more anti-anxiety, exploratory, and pro-social behaviors (Frye et al., 2000c). Systemic administration of 3*α*,5*α*-THP, or MBR agonists which increase 3*α*,5*α*-THP levels, produces anti-anxiety and antipsychotic behavioral effects (Bitran et al., 2001). In contrast, inhibiting formation of 3*α*,5*α*-THP, or antagonizing GBRs, blocks the pro-social, anxiolytic, and antipsychotic effects of 3*α*,5*α*-THP (Rhodes and Frye, 2001). In addition to mitigating fearful responses, results of conditioned place preference and drug discrimination studies suggest that 3*α*,5*α*-THP can also have reinforcing effects (Finn et al., 1997; Russo et al., 2003). Thus, 3*α*,5*α*-THP may mediate responses to stressors encountered during mating by inhibiting negative responses and/or enhancing positive ones.

3α,5α-THP and solicitation behaviors

Progestins facilitate solicitation behaviors. Systemic administration of P or 3*α*,5*α*-THP similarly increases hopping and darting behaviors that female rats make toward male rats to solicit sexual contacts. Co-administration of biosynthesis or metabolism inhibitors with P decreases hopping and darting (Frye et al., 1998) and completely eliminates females' pacing of male sexual contacts (Frye et al., 1998). Similarly, progestin administration enhances, and inhibitors attenuate, movements female hamsters make with their perineum to enable mating (Frye and Rhodes, 2005). These data suggest that 3*α*,5*α*-THP may influence solicitation behaviors made by female rodents.

Pacing increases central 3α,5α-THP

Biosynthesis of 3*α*,5*α*-THP is enhanced by paced mating. Female rats that pace their sexual contacts have significantly higher whole brain 3*α*,5*α*-THP levels than do females mated in standard arenas that cannot pace their sexual contacts or rats that are not mated (Frye et al., 1998).

3α,5α-THP and propinquity

Other indices of approach include choice and duration of time female rats spend in proximity to male or female rats in goal boxes on either end of a T-maze or open field. This propinquity measure controls potential confounds of direct social contact (e.g. vaginocervical stimulation) on sexual responsiveness and/or 3*α*,5*α*-THP biosynthesis (Frye, 2001). Administration of P or 3*α*,5*α*-THP, compared with vehicle, to OVX, E-primed rats significantly increases the amount of time female rats spend in proximity to a male. Co-administration of systemic inhibitors of 3*α*,5*α*-THP to hormone-primed rats attenuates female rats' preference for proximity to a male in the T-maze (Frye et al., 1998). These data suggest that 3*α*,5*α*-THP may influence affiliative behavior of rodents.

3α,5α-THP and aggression

3*α*,5*α*-THP can influence other social behaviors that influence mating, such as aggression. 3*α*,5*α*-THP administration to mice reduces alcohol-induced aggression (Miczek et al., 2003). Preliminary data from our laboratory demonstrate that resident, wild-type, female mice that are administered P (1 mg/kg s.c.), made fewer aggressive overtures toward an intruder (~2) than did vehicle-administered mice (~6). Notably, 5*α*-R knockout mice, administered P or vehicle, make a similar number of aggressive displays (5 and 6, respectively) toward intruders within 2 min and had similar low levels of 3*α*,5*α*-THP. Thus, 3*α*,5*α*-THP may have an inhibitory effect on aggression.

3α,5α-THP and anxiety behaviors

3*α*,5*α*-THP may decrease anxiety behaviors, concomitant with enhancing lordosis, solicitation, approach/avoidance, and attenuating aggressive behaviors. P administration to E-primed rats decreases anxiety behavior on the elevated-plus maze similarly as to what is observed for rats in behavioral estrus that have increased endogenous levels of 3*α*,5*α*-THP (Frye et al., 2000c; Frye and Walf, 2004a). Co-administration of P metabolism inhibitors attenuates progestins' anti-anxiety effects (Rhodes and Frye, 2001) and P-administration does not reduce anxiety behavior of 5*α*-R knockout mice (Frye et al., 2004a). 3*α*,5*α*-THP produced from biosynthesis can also have anti-anxiety effects. MBR agonists produce anxiolysis (Bitran et al., 2001), OVX, P-primed rats exposed to the scent of a predator demonstrate analgesia (Walf and Frye, 2003), and mating increases anti-anxiety behavior (Frye, 2001). All of these situations also produce rapid central elevations in 3*α*,5*α*-THP levels.

Although these data suggest that 3*α*,5*α*-THP facilitates lordosis and other reproductively-relevant behaviors, the findings are limited because they are from separate investigations and some utilized systemic drug regimen. To address this, we have employed a test battery that encompasses different behaviors involved in mating. Receptive female rats typically must leave their burrow to find a mate. Behavior in the open field and elevated-plus maze are used as indices of a female's willingness to overcome anxiety in favor of exploration. Females need to interact with potential mates, rather than conspecifics, for mating to occur. Performance in the T-maze task is used to assess the extent to which a female chooses to be close to a potential mate or another female. The number of non-reproductive social interactions is examined when experimental females are paired with a conspecific. In order to successfully reproduce, a female must engage in mating behavior. Consequently, the last task in our behavioral battery is a mating opportunity in which lordosis is measured. Tissues are then collected for measurement of 3*α*,5*α*-THP levels. Effects of estrous cycle differences, 3*α*,5*α*-THP inhibitors to the VTA of naturally-receptive rats, and 3*α*,5*α*-THP administration to the VTA of OVX, E-primed rats are discussed below.

Endogenous increases in midbrain 3α,5α-THP coincide with enhanced social behaviors

Naturally-receptive rats demonstrate more exploration, less anxiety behavior, enhanced social behaviors and had higher midbrain 3*α*,5*α*-THP levels, than diestrous rats. When tested according to our previously published methods (Frye et al., 2000c), rats in behavioral estrus, compared with diestrus, spent more time: exploring the center of an open field, on the open arms of the elevated-plus maze, in non-physical social interaction with a male in a T-maze, in direct physical contact with a female, and mating (Table 1, left). Although these data are consistent with increases in 3*α*,5*α*-THP in the midbrain during behavioral estrus being associated with more exploration, less anxiety behavior, and greater social interactions, they do not demonstrate causal effects of 3*α*,5*α*-THP.

Inhibiting endogenous increases in midbrain 3α,5α-THP decreases social behaviors

We have investigated causal effects of 3*α*,5*α*-THP in the VTA on social and affective behaviors and the putative sources of 3*α*,5*α*-THP. Naturally-receptive rats were infused with 1 *μ*l of *β*-cyclodextran vehicle, or 1 *μ*g of a biosynthesis inhibitor (digitoxin), metabolism inhibitor (finasteride), or both, to the VTA two hours prior to testing (as in Petralia et al., in preparation). Infusions of a biosynthesis inhibitor, metabolism inhibitor, or both had similar effects to decrease the number of central entries in the open field, the amount of time spent on the open arms of the elevated-plus maze, time spent in proximity to a male, time spent interacting with a conspecific, lordosis quotients, and midbrain 3*α*,5*α*-THP levels compared with vehicle infusion to rats in behavioral estrous (Table 1, middle). Notably, administration of these inhibitors produced a pattern of effects that was analogous to that observed for diestrous rats.

3α,5α-THP infusions to the VTA facilitate socio-sexual behaviors

We have investigated whether effects of 3*α*,5*α*-THP in the VTA are sufficient to enhance social behaviors. OVX rats were E-primed (10 *μ*g 48 h prior to test) and infused bilaterally with 3*α*, 5*α*-THP (100 ng) or vehicle (*β*-cyclodextran) to the VTA 30 min before testing (as in Frye et al., 2004b). 3*α*,5*α*-THP, as compared with vehicle infusions to the VTA, increased central entries in the open field, time spent on the open arms of the elevated-plus maze, duration of time spent in proximity to a male, time spent interacting with a conspecific, lordosis quotients, and midbrain 3*α*,5*α*-THP levels (Table 1, right).

These data suggest that actions of 3*α*,5*α*-THP in the VTA are important for progestin-modulated social and affective behaviors and that biosynthesis of 3*α*,5*α*-THP in the VTA may underlie such effects. Future studies will investigate the extent to which each behavior, rather than the cumulative behavioral battery, can alter biosynthesis of 3*α*,5*α*-THP and also the possible substrates in the VTA, and other CNS sites, such as the hippocampus and prefrontal cortex, for behavioral effects of 3*α*,5*α*-THP.

3α,5α-THP's role in women's health and neuropsychiatric disorders

Elucidating 3*α*,5*α*-THP's effects and mechanisms is important because of its potential role in the etiology and/or treatment of neuropsychiatric disorders.

Sex differences in 3α,5α-THP

In general, females have higher levels of, and greater variations in, 3*α*,5*α*-THP than do males. Womens' 3*α*,5*α*-THP levels are much higher than mens', except during the follicular phase (Pearson-Murphy and Allison, 2000). This same pattern is seen in rats and covaries with reproductive behaviors (Frye and Bayon, 1999). These sex differences in 3*α*,5*α*-THP are mainly due to gonadal and adrenal sources; however, central 3*α*,5*α*-THP levels also vary.

Stress-induced biosynthesis of 3*α*,5*α*-THP is also greater for females than males. Females, as well as males, show stress-induced elevations in 3*α*,5*α*-THP production (Paul and Purdy, 1992; Barbaccia et al., 2001). Further, greater 3*α*,5*α*-THP biosynthesis occurs for females, than males, in response to more modest stressors (McCormick et al., 2002). Perinatal stress may also have more pervasive effects throughout life on females than males and may be mitigated by gonadal status in adulthood (Mitev et al., 2003). Thus, females compared with males may be more vulnerable to stressors, in part due to 3*α*,5*α*-THP.

3*α*,5*α*-THP may alleviate effects of stress, and/or some neuropsychiatric symptoms, through its actions at the HPA. Depression and schizophrenia are often characterized by aberrant stress response and/or diminished activity of the reproductive axis (Guidotti et al., 2001; Rupprecht et al., 2001; Seeman 2002; Young and Korszun, 2002). Given that 3*α*,5*α*-THP mitigates HPA responses, and there are sex differences in 3*α*,5*α*-THP and stress responsiveness, whether 3*α*,5*α*-THP is a neuroendocrine mediator that underlies sex/hormonal differences in responses to behavioral/environmental stimuli is of interest.

3α,5α-THP and mood disorders

Sex differences and/or fluctuations in plasma and/or central concentrations of 3*α*,5*α*-THP may mitigate affective disorders. More women than men have mood disorders, which can be linked to hormonal status (Young and Korszun, 2002). Premenstrual syndrome, postpartum depression, and associated psychoses, are defined by psychiatric disturbances with menstruation or parturition (when 3*α*,5*α*-THP levels decrease precipitously; Freeman et al., 2002; Young and Korszun, 2002). Among women, hospitalization for depression most often occurs perimenstrually (Kolakowska, 1975). Animal studies support these clinical findings. Rats with higher 3*α*,5*α*-THP concentrations (receptive, pregnant), show less anxiety and depressive behaviors than do those with lower 3*α*,5*α*-THP levels (diestrous, postpartum, males) (Frye et al., 2000c; Frye and Walf, 2002, 2004b). Thus, endogenous variations in 3*α*,5*α*-THP may underlie changes in affect.

Therapeutics for mood disorders can alter 3*α*,5*α*-THP. Fluoxetine, a selective serotonin reuptake inhibitor, alleviated depression and restored 3*α*,5*α*-THP levels of depressed men to that of non-depressed controls (Uzunova et al., 1998; Guidotti et al., 2001). Changes in plasma 3*α*,5*α*-THP levels also occurred with beneficial placebo responses among women with premenstrual dysphoric disorder (Freeman et al., 2002). 3*α*,5*α*-THP has anti-depressant effects in animal models (Frye and Walf, 2002, 2004b). 3*α*,5*α*-THP, fluoxetine, or other drugs that increase 3*α*,5*α*-THP decrease depressive behavior (Khisti et al., 2000). Thus, 3*α*,5*α*-THP may be involved in the pathophysiology and/or treatment of depression.

3α,5α-THP and schizoaffective disorders

Sex differences that favor women suggest that 3*α*,5*α*-THP may have a protective role in schizophrenia. Women with schizophrenia experience later age of onset, less debilitating psychiatric symptoms, fewer psychiatric hospitalizations, better pre- and post-functioning, and a more rapid and greater response to drug treatments than do men (Seeman, 2002). Schizophrenic women are more likely to experience first onset or exacerbation of symptoms of psychotic episodes perimenstrually or perimenopausally, when 3*α*,5*α*-THP levels are low (Seeman, 2002). Incidence of a null mutation which disrupts function of MBRs is higher among schizophrenics, than in a control population (Kurumaji et al., 2000). In animal models of schizophrenia, social isolation of mice decreases 3*α*,5*α*-THP levels (Dong et al., 2001). The atypical antipsychotic, olanzapine, increases 3*α*,5*α*-THP levels and enhances social and affective behavior of rodents (Frye and Seliga, 2003). Thus, 3*α*,5*α*-THP may have an important role in schizoaffective disorders.

CONCLUSION

In summary, 3*α*,5*α*-THP may be involved in sex and hormonal influences on social, affective, and stress processes, and the etiology and/or treatment of mood and schizoaffective disorders. The data reviewed here indicate that: 1) 3*α*,5*α*-THP mediates lordosis through actions in the VTA. 2) 3*α*,5*α*-THP has, in separate reports, been demonstrated to alter solicitation, aggression, and anxiety behavior. 3) 3*α*,5*α*-THP in the midbrain VTA facilitates social and affective behaviors in an ethologically-relevant test battery. These findings may be related to others from the literature which indicate that 3*α*,5*α*-THP variations may mediate stress and/or vulnerability to neuropsychiatric disorders.

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Abbreviations:

cAMP, cyclic AMP
D_1, dopamine type 1 receptors
E, estrogen
GAD, glutamic acid decarboxylase
GBRs, $GABA_A$/benzodiazepine receptors
HPA, hypothalamic-pituitary-adrenal axis
MBRs, mitochondrial benzodiazepine receptors
NMDARs, NMDA type glutamate receptors
OVX, ovariectomized
P, progesterone
PRs, progestin receptors
P450scc, P450 side chain cleavage enzyme
VTA, ventral tegmental area
3-HSOR, 3-hydroxysteroid oxidoreductase enzymes
3*α*,5*α*-THP, 3*α*-hydroxy-5*α*-pregnan-20-one
3*β*-HSD, 3*β*-hydroxysteroid dehydrogenase
5*α*-R, 5*α*-reductase

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Table 1

Behavioral changes concomitant with variations in midbrain 3α,5α-THP

Dependent measures	Experimental condition						
	Diestrus	Behavior at estrus	Biosynthesis inhibitor to VTA	Metabolism inhibitor to VTA	Biosynthesis and metabolism inhibitor to VTA	OVX+SC E_2+ vehicle to VTA	OVX+SC E_2+3α,5α-THP to VTA
Central entries[#]	6±1	17±6[*]	0±0[#]	2±1[#]	3±1[#]	13±12	98±8[**]
Open arm time (s)	28±4	110±12[*]	2±2[#]	9±6[#]	8±8[#]	21±12	34±5
Proximity to male (s)	84±13	119±11	16±2[#]	83±12[#]	20±4[#]	40±26	68±12[**]
Social interaction (s)	55±7	92±11[*]	19±3[#]	27±7[#]	28±4[#]	30±4	89±1[**]
Lordosis quotient (%)	10±2	94±2[*]	46±9[#]	50±3[#]	51±9[#]	25±6	75±11[**]
Midbrain 3α,5α-THP (nmol/g)	6±3	25±3[*]	13±1[#]	12±1[#]	11±1[#]	13±6	25±6[**]

All groups represent three to 12 observations per group.

[*] Indicates that analyses of variance (ANOVAs) revealed these parameters were significantly different for rats in behavioral estrous and diestrous.

[#] Represents that ANOVAs show that rats in behavioral estrus that were infused with the biosynthesis and/or metabolism inhibitors (digitoxin and finasteride, respectively) to the VTA are significantly different from rats in behavioral estrous infused with vehicle.

[**] ANOVAs show that ovx, E_2-primed rats with infusions of 3α,5α-THP to VTA are different from those that receive vehicle to the VTA.