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Progesterone effects on the discriminative stimulus, subjective and performance effects of triazolam in healthy, premenopausal women

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Abstract

There is accumulating evidence that sex plays a critical role in drug abuse. Female sex hormones have been shown to affect central nervous system function and modulate the effects of drugs of abuse. For example, GABA_A receptor function is positively modulated by progesterone metabolites. There is evidence from preclinical in vitro and in vivo studies as well as some clinical research suggesting that progesterone and its metabolites may enhance the behavioral effects of benzodiazepines, which also serve as positive modulators of GABA_A receptors. The purpose of this experiment was to determine the independent and combined discriminative stimulus, subjective and psychomotor effects of progesterone and triazolam in healthy adult premenopausal women. Oral micronized progesterone (100 mg), triazolam (0.06, 0.12 and 0.25 mg/70 kg) and placebo were administered to healthy, premenopausal women ($n=9$) under conditions of low circulating sex hormones. Triazolam alone functioned as a discriminative stimulus and produced prototypical sedative-like effects (e.g., performance impairment, enhanced reports of sedative effects). Progesterone alone produced sedative-like effects on several subjective and performance measures and the dose combination effects of progesterone and triazolam on several subjective measures of drug effect were similar to the summation of the two drug effects in isolation. Progesterone did not substitute for or modify the discriminative stimulus effects of triazolam. These results suggest that the parent hormone, progesterone, and triazolam have discordant neuropharmacological mechanisms of action. Additional research is necessary to determine the degree to which neurosteroids influence sex differences in benzodiazepine use and abuse.

Keywords

Progesterone; Neurosteroid; Benzodiazepine; Triazolam; Women's health

Introduction

Neurosteroids [e.g., progesterone, allopregnanolone, tetrahydrodeoxycorticosterone (TH-DOC)] are abundantly present in premenopausal women, with plasma concentrations of progesterone varying as a function of menstrual cycle phase and peaking during the mid-luteal phase (plasma concentrations range from 3 to 30 ng/ml [Sofuoglu et al., 2001]). Both

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pre-clinical and clinical research indicates that neurosteroids modulate the rewarding, discriminative stimulus and performance effects of several drugs of abuse (McAuley et al., 1995; White et al., 2002; Justice et al., 1999; Lynch et al., 2002; Lile et al., 2007; Babalonis et al., 2008). Although genomic effects of sex steroids are well-established, progesterone and its metabolites, allopregnanolone and TH-DOC, also have non-genomic effects at binding sites on neuronal membrane-bound receptors and also modulate the activity of GABA, dopamine, opioid, glutamate and nicotinic acetylcholine receptors (Pluchino et al., 2006; Lena et al., 1993). Both in vitro and in vivo studies, for example, have shown evidence that progesterone metabolites modulate activity at the GABA_A receptor complex, with these neurosteroids acting as ligands at extracellular, steroid-specific recognition sites on GABA_A receptors, having affinities and behavioral effects that are comparable to those of many benzodiazepines (Paul & Purdy, 1992). The neuroactive metabolites also dose-dependently increase the effects of benzodiazepines on GABA-induced Cl⁻ currents, indicating that there is a non-genomic, receptor-level interaction between benzodiazepines and neurosteroids in vitro (Paul & Purdy, 1992; Bertz et al., 1995). As GABAergic drugs (e.g., alcohol, various benzodiazepines) potentiate the abuse-related, sedative and performance-impairing effects of sedative drugs (Licata & Rowlett, 2008; Griffiths & Weerts, 1997), it is possible that progesterone and its metabolites play a significant role in sex differences in sedative drug abuse vulnerability (Fischer & Rowlett, 2011; Kroboth et al., 1985; McAuley et al., 1995; Babalonis et al., 2011).

Pre-clinical research has indicated that menstrual cycle phase modulates the behavioral effects of GABAergic drugs. The discriminative stimulus effects of ethanol in non-human primates are enhanced during the mid-luteal phase, when endogenous progesterone levels are elevated (Grant et al., 1997; Green et al., 1999). Clinical drug discrimination studies have also shown cycle modulation of benzodiazepine behavioral effects, with potentiation of the discriminative stimulus effects of triazolam occurring during the mid-luteal phase (relative to the early follicular phase), in healthy, premenopausal women (Babalonis et al., 2008). However, other studies have reported no cycle phase modulation of the subjective and performance-impairing effects of GABAergic drug effects (i.e., triazolam, ethanol) in premenopausal women (Holdstock & de Wit, 2000; Rukstalis & de Wit, 1999). One potential reason for the inconsistent clinical results could be due to the sensitivity of drug discrimination methodology, as cycle modulation of the discriminative stimulus effects of drugs might be more sensitive to neuropharmacological manipulations than subjective or performance measures (Kelly et al., 2003).

Examining drug effects across the cycle provides information about how variations in the endogenous hormonal milieu modulate drug effects, but does not provide a direct measure of the relative effect of any particular hormone, as changes in several hormones frequently co-occur throughout the cycle. For example, during the mid-luteal phase, progesterone levels increase, but estradiol levels are elevated as well, making it difficult to discern the specific effect of progesterone. To determine the independent effect of progesterone and its primary metabolites, the effects of GABAergic drugs have been examined after exogenous progesterone administration. Administration of oral micronized progesterone enhanced the sedative, memory and performance-impairing effects of intravenous triazolam in post-menopausal women (McAuley et al., 1995). In addition, oral micronized progesterone enhanced the subjective and performance-impairing effects of oral triazolam in healthy, premenopausal women during the early follicular phase, when endogenous progesterone and estrogen are at nadir levels (Babalonis et al., 2011). Those studies provided evidence that acute administration of progesterone enhances some behavioral effects of benzodiazepines; however the magnitude of the interactive effects observed on subjective and performance measures might have been underestimated in those studies, as the onset and peak effects of progesterone occurred prior to triazolam effects, and interactions between progesterone and

triazolam were detected after peak progesterone effects had occurred. Following progesterone administration, neuroactive progesterone metabolites, specifically allopregnanolone, peak more slowly and remain elevated several hours after oral progesterone levels peak (Andréen et al., 2006; Nahoul et al., 1993). As such, it is also possible that drug interactions in the previous studies were driven predominantly by neuroactive progesterone metabolites, rather than the parent hormone, progesterone.

The primary aim of the present study was to determine the independent and combined discriminative stimulus, subjective and psychomotor effects of progesterone and triazolam in healthy adult premenopausal women, using a dosing schedule designed to engender concurrent onset of peak drug and hormone levels. The second aim of this study was to examine the stimulus effects of progesterone, alone and in combination with triazolam, using a drug discrimination methodology. Drug discrimination methodology was included to more precisely examine the behavioral pharmacological effects of progesterone, alone and combination with triazolam, as drug discrimination has been shown to be an effective tool in examining pharmacological changes mediated by receptor-based interactions (Colpaert, 1999; Kelly et al., 2003).

Methods

Participants

Healthy, adult premenopausal women were recruited via local newspaper advertisements and with flyer postings on a university campus. All potential participants completed an initial telephone or internet-based questionnaire and an on-site medical evaluation that included health history and psychological questionnaires, blood chemistry and urinalysis. Urine samples were also screened for drugs of abuse and pregnancy. Eligibility criteria included a minimum of 18 years of age, reports of occasional sedative drug use (e.g., alcohol), and use of an oral, hormone-based contraceptive that contained both estradiol and a progestin and included a 7 consecutive-day placebo phase. Individuals presenting with histories of cardiovascular, neurological, or major psychiatric illnesses, including current or past drug or alcohol abuse or dependence were excluded from participation. Individuals reporting excessive use of alcohol or caffeine, pregnant or breastfeeding status, or peanut allergy (as the progesterone formulation, Prometrium[®], contained peanut oil) were also excluded from study participation.

Fourteen participants were enrolled in the study. Three participants withdrew from the study for reasons unrelated to the study protocol, two were discontinued from the study because they did not acquire the drug discrimination; data from these five participants were not included in the data analyses. Nine participants (8 Caucasian, 1 African American) completed the study. The participants ranged in age from 18 to 30 years (median = 22 years) and in weight from 55 to 93 kilograms (median = 65.95 kg; BMI range = 21 to 31, median = 22.1). All participants were non-smokers. Alcohol use ranged from less than 1 to 8 alcohol drinks per week (median = 4) and caffeine use ranged from 0 to 200 mg of caffeine per day (median = 80 mg). Participants reported no drug use, other than alcohol and caffeine, in the month prior to study participation, and no drug use was detected during the study with daily urinalysis testing. Urine screens for pregnancy were also negative throughout participation. All subjects provided sober, written informed consent. Participant study earnings ranged from \$55 to \$70 per day (approximately \$10/hour). The Institutional Review Board of the University of Kentucky Medical Center approved the study and the informed consent document. The study was conducted in accordance with the ethical standards of the 1964 Declaration of Helsinki (2008).

Design

A double-blind, placebo-controlled, randomized-dose design was used to assess the effects of three within-subject variables: progesterone dose (100 mg), triazolam dose (training dose: 0.25 mg/70 kg, test doses: 0.06 and 0.12 mg/70 kg), and time (assessments occurring prior to and 30, 60, 90, 120 and 150 minutes after dose administration) on the discriminative stimulus and other behavioral effects of triazolam during the early follicular phase of the menstrual cycle (i.e., placebo phase of the oral birth control regimen), when estrogen and progesterone levels are at their nadir.

The 100 mg progesterone dose engenders blood levels of progesterone (in the range of 3 to 13 ng/ml) that approximate the blood levels that are detected during the mid luteal phase of the menstrual cycle (Nahoul et al., 1993; Stanczyk, 1999), and when administered with a moderate-fat snack, produced peak behavioral effects 75 to 105 minutes post-dose (Babalonis et al., 2011). When administered with a moderate-fat snack, peak triazolam effects are expected to emerge 90 to 150 minutes post oral dose administration (e.g., Babalonis et al., 2011); in an effort to examine the concurrent peak effects of these medications, progesterone and triazolam were administered simultaneously.

Schedule

Participants completed one training session prior to the experimental sessions, to acquaint them with the study procedures and to establish consistent and accurate performance on computerized behavioral tasks. This practice session was conducted irrespective of menstrual cycle phase but within one week of the initiation of the experimental sessions.

Participants were instructed to abstain from alcohol and all medications for 24 h and caffeine and food for 4 h prior to their scheduled sessions. At the beginning of each session, participants were asked questions about medication and oral birth control use, sleep, food consumption and health status for the preceding 24 h. No sessions were cancelled due to reports of atypical activities. Participants then completed a field-sobriety test, provided a breath sample that was tested for alcohol use (Alco-Sensor III, Intoximeters, Inc., St. Louis, MO) and provided a urine sample that was tested for amphetamine, barbiturates, benzodiazepines, cocaine, marijuana, methadone, methamphetamine, MDMA, and opiates (E-Z Split Key Cup, ACON Laboratories, San Diego) and pregnancy (hCG One Step Pregnancy Test Device, Instant Technologies, Norfolk, VA).

After a baseline assessment, progesterone and triazolam capsules were administered simultaneously. Fifteen minutes after capsule administration, participants consumed a moderate-fat snack to enhance progesterone absorption (Simon et al., 1993; Stanczyk, 1999). Assessments were repeated at 30-minute intervals for 2.5 h after capsule administration, and consisted of the Drug Discrimination Task (post-dose assessments only), commonly used clinical measures of drug effect [Visual Analog (VAS) and Adjective Rating Scales (ARS), Addiction Research Center Inventory (ARCI), Profile of Mood States (POMS), Digit Symbol Substitution Task (DSST), and heart rate and blood pressure measurement (Sentry II, NBS Medical, Costa Mesa, CA)] and a measure of risk-taking behavior [Balloon Analog Risk Task (BART)]. After all assessments were complete, participants remained at the laboratory for a minimum of two hours to rest and allow drug effects to dissipate. Prior to leaving the laboratory, participants completed the field sobriety test in the same manner as their baseline test and reported no remaining drug effects.

Assessment Tasks

Drug discrimination task—During this task, two circles labelled with a letter code (e.g., “Drug A” and “Not Drug A”) were displayed on the computer screen. Counters were

displayed directly beneath the circles. A mouse controlled the location of a cursor on the screen, and mouse button clicks with a cursor positioned on a circle increased the counter associated with that circle according to a Fixed Interval (FI) 1-s schedule. Participants could alternate responses between circles without any consequence (i.e., no changeover delay). Up to 60 points could be allocated across the two options. The dependent variable for this task was the percent of responding on the drug-appropriate option (i.e., Drug A option). The Drug A vs. Not Drug A drug discrimination methodology is reported to establish discrimination behavior that is consistent with neuropharmacological activity (e.g., Preston & Bigelow, 2000; 1994; Preston et al., 1992).

Sampling, control and testing phases were presented during the study. Sampling and control phases were conducted without regard to menstrual cycle phase. The test phase was scheduled to coincide with the early follicular phase of the menstrual cycle (i.e., placebo phase of the oral birth control regimen), when endogenous levels of both progesterone and estrogen are at nadir levels. Participants completed the Test Phase across 2 to 4 consecutive menstrual cycle phases (e.g., 2 to 4 months).

Sampling Phase: The sampling phase consisted of two experimental sessions. On each of the two sessions, participants were informed that the medication she was receiving was 'Drug A' (0.25 mg/70 kg triazolam). A unique letter code (i.e., 'Drug A') was used for each participant.

Control Phase: The control phase was used to determine whether participants had acquired the drug discrimination. Daily schedules during the control phase were identical to those during the sampling phase, with the exception that the participants were blind to the identity of the administered drug (i.e., Drug A, or Not Drug A) until the end of the session. The training dose of triazolam (0.25 mg/70 kg, Drug A) or placebo (Not Drug A) was administered randomly in combination with placebo progesterone under double-blind conditions, with each condition presented at least twice; the same condition was never administered on more than three consecutive days. The criterion for acquisition of drug discrimination was $\geq 80\%$ of responses allocated to the drug-appropriate option during the last assessment of the session (150 minutes post-dose) for five consecutive sessions. If the acquisition criterion was not met in 12 sessions, participants were dismissed from the study. Participants who met criteria did so within 5 to 12 sessions. Performance payment was contingent on discrimination responding, with 10 cents earned for each correct response (i.e., points) allocated to the option associated with the dose administered that day.

Test Phase: During the Test Phase, dose-response generalization functions were established for triazolam (0.06, 0.12, and 0.25 mg/70 kg) alone and in combination with progesterone (100 mg). One permutation of all possible combinations of progesterone (100 mg), triazolam (0.06, 0.12, and 0.25 mg/70 kg) and placebo was administered during an experimental session in random order, for a total of 8 sessions. On days that participants received combinations presented during the Control Phase (i.e., placebo or training doses: 0.00 or 0.25 mg/70 kg oral triazolam with 0.00 mg progesterone), participants received feedback regarding the accuracy of their performance at the end of the session and payment was contingent upon accuracy (as in the Control Phase). On test drug combination days, participants received the average earnings from placebo or training dose days, regardless of discrimination responding.

Visual Analog Scale (VAS)—Participants rated 32 items presented individually on the computer by marking a 100-unit line anchored on the extremes by "Not At All" and "Extremely". VAS items included Stimulated, Stressed, Sedated, Hungry, Thirsty, Anxious, Nervous, Light-Headed, Sleepy, Restless, Sick to Stomach, Talkative, Friendly, Shaky,

Jittery, Down, Depressed, High, Euphoric, Irregular/Racing Heart Beat, Active, Alert, Energetic, Rush, Drug Effect, Good Drug Effect, Bad Drug Effect, Performance Impaired, Performance Improved, Like Drug, Willing to Take Drug Again, and Pay for Drug.

Addiction Research Center Inventory (ARCI)—The 49-item short form of the true-false inventory (Martin et al., 1971) yielded information on five dimensions: Lysergic Acid Diethylamide (LSD) scale, Amphetamine (A) Scale, Benzedrine-Group (BG) Scale, Morphine-Benzedrine Group (MBG) Scale and the Pentobarbital, Chlorpromazine, Alcohol Group (PCAG) Scale.

Adjective Rating Scale (ARS)—The ARS consists of 32 items and contains two subscales: Sedative and Stimulant (Oliveto et al., 1992). In the present study, only the 16 items from the Sedative subscale were presented. Participants rated each item using a numeric keypad to select one of five options: “Not at All”, “A Little Bit”, “Moderately”, “Quite A Bit” and “Extremely” (scored numerically from 0 to 4, respectively; maximum score =64).

Digit Symbol Substitution Task (DSST)—Participants completed a 2 minute computerized version of the DSST adopted from McLeod et al., 1982. Performance on this task is sensitive to the sedative effects of drugs and alcohol (Greenblatt et al., 2005; Jackson et al., 2005). Subjects earned \$0.02 for each correct trial completed. The dependent measures for this psychomotor task were trial completion rate and accuracy.

Balloon Analog Risk Task (BART)—Participants were presented with 20 individual balloons in succession on a computer screen, as adapted from Lejuez et al., 2002. Participants clicked a mouse to inflate each balloon. Each inflation increased the balloon earnings counter by \$0.01 and increased the probability that the next inflation would pop the balloon. Each balloon would pop after an unpredictable number of inflations. Participants could collect their balloon earnings prior to a balloon popping by clicking on a “Collect Money” option. However, if a balloon popped, earnings from the balloon were permanently lost. Earnings from each task presentation were recorded and participants were given their task earnings from a randomly-chosen assessment at the end of the session. The dependent measures were number of inflation responses per un-popped balloons, and number of balloons popped.

Drugs

Doses of progesterone and triazolam were prepared by the University of Kentucky Investigational Pharmacy. Progesterone (0 and 100 mg; Prometrium®) and triazolam (0.00, 0.06, 0.12 and 0.25 mg/70 kg) were prepared in size 00 and 0 distinct opaque capsules, respectively, each with cornstarch filler. Placebo capsules contained only cornstarch filler. Both progesterone and triazolam capsules were delivered orally.

Data Analysis

Drug discrimination data (time points 90 through 150 minutes post-dose) were analyzed using repeated-measures ANOVA, with triazolam dose and progesterone dose as factors. Subjective and performance measures were analyzed using repeated-measures ANOVA with triazolam dose, progesterone dose and time as factors. Main effects were analyzed using Tukey HSD pairwise comparisons, and significant interactions were evaluated using simple-effects analyses.

RESULTS

Drug Discrimination

Figure 1 presents mean drug-appropriate responding as a function of triazolam and progesterone dose during control and test sessions. During control sessions, participants met the drug discrimination acquisition criterion in a mean of 7 sessions (range of 5 to 12, median = 8). Across the final 5 control sessions, participants emitted 0 % and 99% drug-appropriate responding 150 minutes after placebo and active drug administration, respectively. During the test phase, training dose conditions (0.00 and 0.25 mg/70 kg triazolam) engendered 11% and 100% triazolam-like responding. The triazolam test doses (0.06 and 0.12 mg/70 kg) produced intermediate triazolam-like responding (30% and 50%, respectively). This intermediate responding was due to averaging data from participants identifying the dose as either drug-like or non-drug-like (i.e., quantal responding by individual subjects). Progesterone alone engendered 27% drug-like responding (resulting from two participants identifying 100 mg progesterone as drug-like and seven identifying it as not drug like). Progesterone in combination with triazolam did not modify drug discrimination responding at any triazolam dose.

Subjective Effects—Triazolam alone produced sedative-like effects on multiple self-report measures, including significant increases in ARS Sedation ($F[3,24]=5.47, p < .05$), VAS Sedated ($F[3,24]=4.41, p < .05$) and Performance Impaired ($F[3,24]=5.90, p < .05$), POMS Fatigue ($F[3,24]=3.29, p < .05$) and ARCI PCAG Scale ($F[3,24]=5.46, p < .05$). In addition, triazolam significantly increased ratings of VAS Light-Headed ($F[3,24]=1.82, p < .05$), Sleepy ($F[3,24]=2.23, p < .05$), High ($F[3,24]=4.28, p < .05$), and Drug Effect ($F[3,24]=8.29, p < .05$) and significantly decreased ratings of VAS Energetic ($F[3,24]=2.11, p < .05$) and POMS Vigor ($F[3,24]=2.12, p < .05$) and Arousal ($F[3,24]=2.24, p < .05$). Figure 2 presents VAS ratings of Sedated (top row), Performance Impaired (middle row) and Drug Effect (bottom row) as exemplars. Peak effects of triazolam occurred 60 through 90 minutes after administration on each measure.

Independent of triazolam, progesterone also engendered sedative-like effects, including significant increases in VAS Sedated ($F[1,8]=7.75, p < .05$), Sleepy ($F[1,8]=7.28, p < .05$) and Performance Impaired ($F[1,8]=5.86, p < .05$). In addition, progesterone also produced significant increases in VAS ratings of Drug Effect ($F[1,8]=2.77, p < .05$) and Good Drug Effect ($F[1,8]=2.72, p < .05$). Peak effects of progesterone also occurred 60 to 90 minutes after administration on each measure.

The combination of progesterone and triazolam engendered small magnitude increases in subjective effects, compared to either dose alone (Figure 2). The effects of progesterone on the response to triazolam were equivalent to the summation of the two drug effects in isolation, and there was no statistical interaction between progesterone and triazolam. In addition, progesterone delayed the time at which peak effects occurred for the high dose of triazolam (0.25 mg/70 kg; 90 through 150 minutes).

Performance Effects—Triazolam produced sedative-like impairment on measures of psychomotor task performance, with a main effect of triazolam detected on DSST trial rate ($F[3,24]=13.48, p < .05$) and accuracy ($F[3,24]=3.54, p < .05$). Independent of triazolam, progesterone decreased trial accuracy ($F[1,8]=7.65, p < .05$). Similar to what was observed for the combined effects of progesterone and triazolam on subjective effects, the dose combination effects were similar to the summation of the two drug effects in isolation.

No independent or combined effects of progesterone or triazolam were observed on BART performance.

Cardiovascular Effects—Significant triazolam and progesterone effects were observed on diastolic blood pressure ($F[3,24]=2.86$; $F[1,8]=7.20$, $p<.05$); however, these effects were small in magnitude (approximately 5 mm Hg) and occurred intermittently. It is unlikely that these changes represented systematic drug effects or drug interactions.

Discussion

The present study used drug discrimination and subjective, performance and cardiovascular measures to examine the effects of progesterone, alone and in combination with triazolam, in healthy, premenopausal women. Consistent with previous studies, triazolam alone engendered a broad range of sedative-like performance impairment and self-reported effects, with peak effects occurring 60 to 90 minutes post dose (Greenblatt et al., 2005; Babalonis et al., 2008; Rush et al., 2003; Simpson & Rush, 2002; Rush & Ali, 1999). Progesterone alone also produced small magnitude increases in sedative-like measures with a similar time course. The combination of progesterone and triazolam engendered subjective and performance-impairing effects that were similar to the summation of the two drug effects in isolation and delayed the time at which the peak effects of the 0.25 mg/70 kg dose of triazolam occurred by approximately 30 to 60 minutes. Triazolam functioned as a discriminative stimulus, but progesterone pre-treatment did not consistently substitute for, or modulate, the discriminative stimulus effects of triazolam.

This study had two aims, the first of which was to examine the effects of progesterone and triazolam under conditions in which the peak effects for the hormone and drug occurred at similar times. To accomplish this goal, progesterone and triazolam were administered simultaneously, as previous research has indicated that peak effects emerge at similar times (Babalonis et al., 2011). This approach was successful, as peak effects of both progesterone and triazolam occurred 60 to 90 minutes post-dose. Worth mentioning is that self-reported and performance-impairing effects of progesterone alone are not typically observed unless significantly higher oral doses (600 to 1200 mg) or routes of administration with greater bioavailability (i.e., intramuscular administration) are used (Freeman et al., 1993; Soderpalm et al., 2004; de Wit et al., 2001; van der Meer et al., 1982). These results replicate a recent study in which doses of progesterone (100 and 200 mg PO) also increased ratings of several sedative-like measures and engendered psychomotor task performance impairment (Babalonis et al., 2011). The effects of progesterone may have been enhanced in that study, and the present study, due to the administration of moderate-fat snack 15 minutes after progesterone administration, which has been reported to increase progesterone bioavailability (Simon et al., 1993; Stanczyk, 1999). The effects of the combination of triazolam and progesterone were determined under conditions in which the onset of peak drug and hormone levels were concurrent when administered alone. The combination of progesterone and triazolam engendered subjective and performance-impairing effects similar to the summation of the two drug effects in isolation, but not at the time of peak effects of progesterone and triazolam. Combination drug effects occurred 30 to 60 minutes after peak effects of triazolam were observed in the absence of progesterone. These results suggest that the drug interactions might have been driven by neuroactive progesterone metabolites, rather than the parent drug, progesterone.

The second aim of this study was to examine the neuropharmacological effects of progesterone, alone and combination with triazolam, using a drug discrimination methodology. Progesterone alone did not substitute for the training dose of triazolam and concurrent progesterone administration did not alter the discriminative stimulus effects of triazolam. Given that substitution and pretreatment effects are well-established outcomes given common receptor-based pharmacological interactions (Colpaert, 1999; Kelly et al., 2003), these data suggest distinct receptor-mediated pharmacological mechanisms of action

that contribute to the stimulus effects of progesterone and triazolam. For example, one possibility is that the sedative effects of progesterone were not mediated by a GABAergic mechanism. While in vitro and pre-clinical in vivo research has clearly documented GABAergic interactions between neurosteroids and GABAergic agents, progesterone and its metabolites have also been shown to modulate the activity of dopamine, serotonin, opiate, glutamate and nicotinic acetylcholine receptors (Pluchino et al., 2006; Lena et al., 1993). However, other interpretations are also plausible. First, only a single, relatively small dose of progesterone was tested. Progesterone might have engendered small, GABA-mediated increases in the effects of the low doses of triazolam that were below the threshold necessary to produce reliable drug-like responding on the drug discrimination task. Second, it is possible that progesterone modulation of triazolam effects is dependent on triazolam dose (Green et al., 1999). There is some evidence to support this possibility in that progesterone effects on the highest triazolam dose (i.e., a shift in the time course of the peak effects of the 0.25 mg/70 kg training dose) were distinct from those occurring at lower doses. Because this shift was limited to the training dose of triazolam, an enhancement would not have been detected due to the ceiling effect imposed by the drug discrimination procedure. A third possibility is that progesterone modulation of triazolam effects is dependent on progesterone metabolites, rather than progesterone per se (Lundgren et al., 2003; Majewska et al., 1986). In support of this notion, the shift in peak effects of the highest triazolam dose occurred after progesterone effects had dissipated and progesterone metabolites, such as allopregnanolone, would be expected to peak. Specifically, allopregnanolone levels increase 2 hours after oral progesterone administration and remain elevated for up to 8 hours (Andréen et al., 2006; Nahoul et al., 1993). The interactions between progesterone and triazolam documented in previous studies also occurred at times corresponding to the emergence of progesterone metabolites (McAuley et al., 1995; Babalonis et al., 2011). Lastly, the shift in peak effects could be explained with equal plausibility by a pharmacokinetic interaction between triazolam and progesterone. Future research would be required to examine these possibilities.

The drug discrimination results of this study were not consistent with those of a previous study demonstrating that the discriminative stimulus effects of triazolam were potentiated during the mid luteal phase, when endogenous progesterone levels were elevated (Babalonis, et al., 2008). Several factors could have contributed to these disparate results, including the presence of other neurosteroids, the duration of exposure to neurosteroids, progesterone delivery and the discriminative stimulus training conditions. The current study used exogenous, oral progesterone, while the previous study relied on naturally occurring changes in endogenous progesterone levels across the menstrual cycle. The progesterone dose used in the current study was selected to engender systemic progesterone levels comparable to those of the mid luteal phase (Simon et al., 1993; Stanczyk, 1999). However, during the mid luteal phase, there is a broader milieu of hormones present, including estrogen and progesterone metabolites, such as allopregnanolone (Genazzani et al., 1998). The relative influence of estrogen on GABAergic activity is not clear, as estrogen has been reported to positively (Frye & Rhoades, 2005) and negatively (Becker & Hu, 2008) modulate GABAergic activity in pre-clinical models. However, allopregnanolone is neuroactive and a ligand at the GABA_A receptor, and several cell culture and preclinical studies have identified allopregnanolone as a key component in the hormone-benzodiazepine interaction at the GABA_A receptor (Lambert et al., 2009; Paul & Purdy, 1992). The duration of exposure to neurosteroids may also have influenced sensitivity to the discriminative stimulus effects of triazolam across studies. During the luteal phase of the menstrual cycle, progesterone and allopregnanolone levels remain elevated for approximately 10 days (Genazzani et al., 1998). This cycle-related chronic exposure to neurosteroids might engender a genomic-based sensitivity to benzodiazepines (Smith et al., 1998), which could have influenced the mid luteal phase enhancement of the discriminative stimulus effects of

triazolam. A third factor that could have resulted in differential effects across studies is progesterone delivery. In the current study, progesterone was administered orally, whereas the previous study relied on endogenous changes in progesterone levels across the menstrual cycle. Several studies have documented inconsistent absorption of progesterone associated with oral dosing, with a 40 fold magnitude of differences in absorption occurring across individuals (McAuley et al., 1996; Simon et al., 1993). However, in the absence of hormone blood levels in the current study, it is difficult to determine the degree to which progesterone was absorbed.

One caveat of the present study that should be mentioned is that sample and control sessions were conducted without regard to menstrual cycle phase. We used this design because drug discrimination training often takes up to 14 sessions to complete and training would span several months if sessions were only conducted during a specific cycle phase. We have used a similar design in previous studies (e.g., Lile et al., 2007; Babalonis et al., 2008) where participants completed discrimination training during various times throughout their cycles and this did not overtly impact individual differences in acquisition of drug discrimination. In addition, this methodology did not appear to affect sensitivity to cycle modulation of the discriminative stimulus effects of triazolam during the test phase, or the impact of exogenous estradiol on the discriminative stimulus effects of d-amphetamine.

Future studies examining a wider range of progesterone pre-treatment doses and progesterone metabolites in combination with both higher and lower training doses of triazolam and longer-acting benzodiazepines would be useful to further examine receptor-based interactions of progesterone and benzodiazepines. In addition to the basic science information obtained from such studies, examining the neurobiological factors that modulate GABAergic drug effects could have important clinical health implications. The abuse-related, sedative and performance impairing effects of benzodiazepines are potentiated by the presence of alcohol, other benzodiazepines or other GABAergic drugs. Both exogenous progesterone (in the form of oral birth control or hormone replacement therapy) and endogenous neurosteroids (present in varying concentrations across the menstrual cycle phase) have also been shown to enhance the sedative and performance impairing effects of benzodiazepines, (Kroboth et al., 1985; McAuley et al., 1995; Babalonis et al., 2011), suggesting that the abuse-related effects of GABAergic drugs in women could also be affected by these neurosteroids. As such, a greater understanding of the neuropharmacological effects of neurosteroids could be important in the development of gender-specific drug abuse treatment and prevention strategies.

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Figure 1.

Mean triazolam-appropriate responding as a function of triazolam dose and progesterone pre-treatment condition from assessments occurring at 150 minutes post-dose during the control and test phases. Data points represent means of nine participants. Uni-directional error bars represent 1 S.E.M.

Figure 2. Mean ratings of VAS Drug Sedated (top row), VAS Performance Impaired (middle row) and VAS Drug Effect (bottom row) as a function of progesterone dose, triazolam dose and time following drug administration. The left column presents triazolam effects after 0 mg progesterone pre-treatment and the right column presents triazolam effects after 100 mg progesterone pre-treatment. Between-subject error bars are omitted for clarity.