Published in final edited form as:

Psychoneuroendocrinology. 2016 March ; 65: 44–53. doi:10.1016/j.psyneuen.2015.12.008.

Effects of progesterone stimulated allopregnanolone on craving and stress response in cocaine dependent men and women

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Abstract

Objectives—Fluctuations in progesterone levels during the menstrual cycle have been shown to affect physiological and subjective effects of cocaine. Furthermore, our laboratory has demonstrated that following drug-cue exposure, cocaine dependent women with high levels of circulating progesterone display lower diastolic and systolic blood pressure responses and report lower levels of anxiety and drug craving compared to cocaine dependent women with low levels of progesterone. In the current study we examined the role of the progesterone derived neuroactive steroid allopregnanolone (ALLO) on stress arousal, inhibitory control and drug craving in cocaine dependent subjects.

Methods—Plasma levels of ALLO were measured using GC/MS in 46 treatment-seeking cocaine dependent men and women on day 5 of a 7-day treatment regimen of micronized progesterone (15M / 8F) (400mg/day) or placebo (14M / 9F) administered in a double blind, randomized manner. As a control, levels of the testosterone derived neurosteroid androstenediol (ADIOL) were also measured. All subjects participated in laboratory sessions on days 5–7 of progesterone/placebo administration in which they were exposed to a series of 5-min personalized guided imagery of either a stressful situation, cocaine use or of a neutral setting and dependent variables including subjective craving, mood, Stroop task as a measure of inhibitory control performance and plasma cortisol were assessed. Participants were grouped by high or low ALLO level and levels of dependent variables compared between ALLO groups.

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Conflict of interest The authors declare that they have no competing financial interests pertaining to the aims and results of this study.

Results—Progesterone relative to placebo significantly increased ALLO levels with no sex differences. There were no effects of micronized progesterone on the testosterone derived ADIOL. Individuals in the high versus the low ALLO group showed decreased levels of cortisol at baseline, and a higher cortisol response to stress; higher positive mood scores at baseline and improved Stroop performance in the drug-cue and stress conditions, and reduced cocaine craving across all imagery conditions.

Conclusions—As expected, cocaine dependent individuals administered progesterone showed significantly higher ALLO plasma levels. High levels of ALLO appeared to normalize basal and stress response levels of cortisol, decrease cocaine craving and also contribute to improvements in positive emotion and Stroop performance in response to stress and drug-cue exposures. These findings suggest that the neuroactive steroid ALLO plays a significant role in mediating the positive effects of progesterone on stress arousal, cognitive performance and drug craving in cocaine dependence.

Keywords

progesterone; allopregnanolone; cocaine dependence; stress; drug cue; craving

1. Introduction

Cocaine dependence is a common and serious health problem (SAMHSA 2007) for which there are currently no approved medications. Early abstinence from cocaine is marked by dysregulated basal and stress-induced physiological, hypothalamic-pituitary-adrenal (HPA) axis and emotional changes, which are strongly associated with increased cocaine craving, cocaine use and relapse (Back et al., 2005; Sinha et al., 2006; Fox et al., 2008a; Fox and Sinha, 2009). As sex steroid hormones play a key role in modulating these relapse-related stress responses, we aim to examine the underlying mechanism more clearly by assessing the role of allopregnanolone (ALLO) in ameliorating stress-induced craving in early abstinent cocaine dependent men and women.

Preclinical and clinical research has shown that fluctuations in endogenous progesterone levels during the menstrual cycle can affect physiological and motivational aspects of cocaine, both following acute intake of the drug, as well as during early recovery from cocaine (for review see Festa and Quinones-Jenab, 2004). For example, cardiovascular and positive-subjective responses to cocaine in women were reduced during the luteal phase of the menstrual cycle, i.e. when progesterone levels are high, compared to the follicular phase when progesterone levels are low (Sofouglu et al., 1999; Evans et al., 2002; Evans and Foltin, 2006). Previous research from our laboratory also showed that women with high levels of circulating progesterone demonstrated lower diastolic and systolic blood pressure responses to cue and reported lower levels of anxiety and drug craving compared to women with low endogenous levels of progesterone (Sinha et al., 2007). Similarly, cocaine dependent women in their first month of abstinence demonstrated significantly up-regulated salivary cortisol and negative affect alongside enhanced levels of progesterone across the entire menstrual cycle, suggesting a potentially compensatory role for endogenous progesterone in response to an enhanced distressed state (Fox et al., 2008b). New clinical

evidence also suggests some benefit in cocaine use outcomes from progesterone treatment in post-partum women with a cocaine use disorder (Yonkers et al. 2014).

This therapeutic effect of progesterone may be mediated by the neuroactive steroid (3 α , 5 α)-3-hydroxypregnan-20-one (a.k.a. allopregnanolone; ALLO), which is synthesized from progesterone. ALLO potentiates the GABA induced opening of the GABA_A receptor chloride channel at nanomolar concentrations, and produces anxiolytic, anticonvulsant and hypnotic effects similar to those induced by other GABA_A receptor potentiating drugs (Lambert et al., 2001; Majewska et al., 1986). Most notably, fluctuations in peripheral and brain ALLO levels have been associated with motivational mood-related changes during pregnancy, menopause, and a number of psychiatric disorders, including depression, premenstrual dysphoric disorder, schizophrenia and bipolar disorder (Barbaccia et al., 1996; Concas et al., 1998; Girdler et al., 2001; Marx et al., 2006; Uzunova et al., 2006).

Recent evidence suggests that progesterone derived neuroactive steroids could also be important in mediating regulatory cognitive function (Milivojevic et al., 2014b) and stress-associated relapse, as supported by the finding that the HPA axis is largely under GABAergic control (Herman et al., 2004). Acute stress has been shown to down-regulate GABA-ergic transmission (Biggio et al., 2007) and increase neuroactive steroid levels in the brain and periphery (Purdy et al., 1991). In addition, intraperitoneal (i.p.) injections of CRF and ACTH in rats increase brain and plasma levels of allopregnanolone (Torres et al., 2001). These findings indicate that neuroactive steroids may play a crucial compensatory role in mediating homeostasis in response to stress (Biggio et al., 2007; Crowley and Girdler, 2014; Sarkar et al., 2011).

In support of this, preclinical studies have found that ALLO administration decreased cocaine primed reinstatement in female, but not in male rats (Anker et al., 2009). In addition, ALLO administration decreased yohimbine-induced cocaine reinstatement in female rats only (Anker and Carroll, 2010), suggesting that ALLO may attenuate stress-induced reinstatement of cocaine seeking in females. More extensive research has examined the role that neuroactive steroids play in alcohol's effects (Morrow et al., 1999; Morrow et al., 2001). For example, acute alcohol increases levels of neuroactive steroids in plasma (VanDoren et al., 2000) and brain (Sanna et al., 2004), and blockade of neuroactive steroid production by the 5 α -reductase (5 α -R) inhibitor finasteride attenuated acquisition of alcohol preference in mice (Ford et al., 2008). In humans, the plasma concentration of ALLO was increased following severe intoxication (Torres and Ortega, 2003, 2004), but not moderate intoxication (Holdstock et al., 2006; Nyberg et al., 2005; Pierucci-Lagha et al., 2006). Importantly, chronic alcohol exposure reduces both plasma and brain levels of ALLO (Morrow et al., 2001). Clinical studies have also demonstrated that variation in genes that encode neuroactive steroid synthesis enzymes was associated with alcohol dependence (Milivojevic et al., 2011) and subjective effects of alcohol (Milivojevic et al., 2014a), which may modulate risk for the development of dependence. Moreover, neuroactive steroids may be mediating nicotine effects, as nicotine has been found to increase neuroactive steroids (Porcu et al., 2003). Unlike alcohol dependence, their levels were found to be higher in smokers, in whom levels of allopregnanolone positively correlated with nicotine dependence

severity (Marx et al., 2006). The role that neuroactive steroids may play in cocaine effects in humans is still largely unclear and has not been systematically examined.

In a previous study we showed that administration of 400 mg/day of exogenous progesterone across 7 days demonstrated some selective efficacy in attenuating cortisol response and cocaine craving following drug cue as well as negative mood in women (Fox et al., 2013). However, the reason for these effects of progesterone are not clear and the extent to which ALLO was a contributing element, is unknown. Moreover, it is not clear whether ALLO has modulatory effects on inhibitory cognitive function. Thus, we examined the effects of the progesterone metabolite ALLO in a larger group of the cocaine dependent individuals recruited for this study (Fox et al., 2013) in order to assess this further. ALLO levels were assayed on day 5 of the 7-day progesterone/placebo treatment regimen to generate high and low ALLO groups. Based on existing preclinical findings, we hypothesized that high ALLO relative to low ALLO will improve mood and inhibitory cognitive control, as assessed using the Stroop task, and decrease cocaine craving and stress response during acute stress and drug cue imagery exposure in a controlled laboratory experiment with progesterone versus placebo treated cocaine dependent men and women. Androstenediol (ADIOL), a neuroactive steroid derived from testosterone was assessed as a control measure to ensure that any observed effects were specific to progesterone stimulated increases in ALLO and not other potent neuroactive steroids, such as the testosterone derived ADIOL. We predicted that ADIOL will not be different by ALLO groups and hence will not have an impact on stress, drug craving and inhibitory control in CD subjects in this laboratory paradigm.

2. Methods

Participants: Forty six treatment-seeking cocaine dependent individuals (29 M/ 17F) were recruited through local advertisements. All subjects from Fox et al., (2013) were included in the current analysis, and the current sample included 4 more participants over the parent study. Participants met DSM-IV criteria for current cocaine dependence and tested positive for cocaine in urine toxicology screens upon entry into inpatient treatment and study at the Clinical Neuroscience Research Unit (CNRU) of the Connecticut Mental Health Center (CMHC) for a 3–4 week inpatient stay. The CNRU is a locked inpatient treatment facility with no access to alcohol or drugs and very limited access to visitors. Alcohol and drug testing was conducted 3 times per week, to ensure drug abstinence. Participants were excluded if they met criteria for any other current psychiatric disorder or current and/or lifetime dependence on substances other than cocaine, alcohol or nicotine, determined using the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders IV (SCID IV; First et al, 1997). Women were excluded from the study if they were using any birth control or were peri- or postmenopausal. All participants were excluded if they required any prescribed medications or were not in good health. All participants gave both written and verbal informed consent and the study was approved by the Human Investigation Committee of the Yale University School of Medicine.

Progesterone/Placebo Administration as an Experimental Probe to Increase ALLO: A

placebo-controlled, double-blind experimental design was used in this study. Micronized progesterone (Prometrium®) (200 mg capsules) were purchased from Solvay Pharmaceuticals, Marietta, Georgia, through the CMHC research pharmacy which then prepared identical appearing progesterone and placebo capsules. Placebo pills contained lactose and abstinent cocaine dependent subjects were randomly assigned to placebo or progesterone conditions by the research pharmacist, and balanced on age, sex, education level and cocaine use severity using Urn randomization procedures (Stout et al., 1994). Women were only admitted to the inpatient unit one to two weeks before their expected beginning of menstruation (menstrual cycle day 1). For a description on how the menstrual cycle was measured, see below. The 7 day medication protocol was initiated between days 1–9 of their menstrual cycle when both progesterone and estradiol levels are low and remain stable (approximately <85 pg/ml for estradiol and <1.1 ng/ml for progesterone; Chabbert-Buffet et al., 1998). In the case of male participants, treatment medication was administered after approximately 7–10 days of inpatient stay for consistency. Study medications were administered at 7:30 am and 9 pm daily for seven days.

Menstrual Cycle History: All women were required to complete a detailed menstrual cycle history questionnaire providing self- report information regarding the dates and regularity of their last three menstrual cycles, the length of menses each time, and the onset of menses in the current cycle. Menstrual cycle phase was confirmed by obtaining serum levels of progesterone and estradiol upon admission to the inpatient unit and during the three laboratory testing days. Serum levels and self- reported menstrual cycle details were then compared to validate menstrual stage.

Safety and Side Effects Measures—All patients were evaluated on a) sitting and standing vital signs (HR/BP) taken every 15 minutes for one hour, every other day while on the medication, b) assessment of laboratory chemistry at study admission and discharge, including serum renal, hepatic, and pancreatic tests, and c) assessment of any changes in physical and mental functioning every other day while on the medication, to identify and evaluate any potential adverse events or side effects. A detailed description of progesterone administration safety and side effects is outlined in Fox et al. (2013).

Acute Stress and Drug Craving Experimental Procedures: ALLO levels were assessed on day 5 of the progesterone/placebo regimen and all subjects were prepared 3 experimental sessions scheduled during days 5–7 of study medication period on consecutive days where subjects were exposed to 5-minute audiotaped scripts of stress, cocaine-related, and neutral, relaxing scenarios, using a well-established and widely used, individually calibrated guided imagery procedure (Sinha et al. 2009). This involved, script development for the personalized stress, drug cue and neutral relaxing imagery provocation, followed by an imagery training and acclimatization session and a 3-day laboratory experiment for stress and drug craving provocation.

a. Imagery development and training: A guided imagery induction method was used in the present study to expose individuals to stress, drug-cues and neutral relaxing control cues. The method involves “reliving” recent stressful, drug-related and neutral relaxing personal

events on separate testing days through a guided imagery procedure. During the second week of inpatient stay, participants took part in the script development session with a member of the research team who was extensively trained on the procedure using the structured imagery procedures manual (Sinha & Tuit, 2012). The stress imagery script was based on each individual subjects' description of a recent personal stressful event that was experienced as most stressful. Examples of highly stressful situations include loss of a job, conflict with a significant other, or loss of an important relationship. Most stressful was determined by having each subject rate their perceived stress on a 10-point Likert scale where 1 = not at all stressful and 10 = the most stress they felt recently in their life. Only situations rated as 8 or above were accepted as appropriate for script development. The stress imagery scripts did not include scenarios either relating to or culminating in drug use. The cue imagery script was developed by having subjects identify a recent situation that included cocaine-related stimuli and resulted in subsequent cocaine use (i.e. watching others smoke crack). Drug-related scenarios did not include scenarios that involved stressful events such as being arrested. A relaxation imagery script was also developed from the subjects' description of a personal non-drug-related relaxing situation, such as a summer beach scene or a relaxing fall afternoon reading. This resulted in three personalized scripts (stress, drug-cue, relaxing). The three scripts were then recorded on audio tape by a trained member of the research team for guided imagery in the laboratory sessions. These procedures have been previously shown to reliably produce drug craving and physiological arousal in drug dependent individuals (Sinha et al., 2000). Full procedures for script development are described in Sinha et al., (2003), Sinha (2009) and Sinha & Tuit (2012).

b. Habituation and Acclimatization: In addition to the script development, on the day prior to the laboratory sessions, subjects were brought into the testing room in order to acclimatize themselves to specific aspects of the study procedures. Specifically, a nurse inserted an IV line into the subject's non-preferred arm without drawing any blood, and subjects were familiarized with the subjective rating forms. Lastly, subjects received standardized and detailed training in relaxation and imagery procedures, during which they were trained in muscle relaxation and how to actively participate in imagining situations as vividly as possible by practicing on 4 different standardized scenarios.

c. Laboratory Sessions: The three imagery conditions (stress, drug-cue, relaxing) were conducted across days 5-7 of study medication on three consecutive days. Conditions were randomized and counterbalanced across participants. Research staff who conducted the experimental sessions was blind to the stimulus condition presented on each day. All subjects who were regular daily smokers were allowed an initial smoke break at 7:30 AM in order to reduce potential nicotine withdrawal during the session. Subjects were allowed four daily smoke breaks during which time they were permitted to smoke 1-2 cigarettes per occasion in order to reduce potential nicotine withdrawal during the study. At 8:00 AM, after settling in a sitting position on a hospital bed, a heparin-treated catheter was inserted by the research nurse in the antecubital region of the subject's non-preferred arm, to periodically obtain blood samples. A blood pressure cuff was placed on the subject's preferred arm and a pulse sensor was placed on the subject's forefinger. This was followed by a 45 minute adaptation period during which the subjects were instructed to practice

relaxation. At 9:10 AM, subjects were provided headphones and given the following instructions for the 5-minute imagery procedure: “Close your eyes and imagine the situation being described, ‘as if’ it were happening right now. Let your body and mind get completely involved in the situation, doing what you would do in the real situation”. All dependent variables (self-reports of drug craving and mood, blood pressure, and blood draws) were obtained at time points before imagery (Pre-Imagery Baseline), immediately after (Imagery) and five times until 75 minutes after imagery (five Recovery points). For an illustration of the laboratory session procedure, see Figure 1. At the end of each session, relaxation instructions were provided to ameliorate any residual effects of imagery exposure. Subjects were then free to leave the testing room and eat breakfast.

2.1. Assessments/Measures

Cocaine Craving Questionnaire-Brief (CCQ; Tiffany et al., 1993): The CCQ (brief) is a shortened adaptation of the 45 item Tiffany Cocaine Craving self-report questionnaire. All 7 items are in the form of a craving-related statement and scores for each item response range from 1 (strongly disagree) to 7 (strongly agree). Differential Emotion Scale (Izard, 1972): The scale comprises 30 adjectives (or items) and participants are required to rate on a 5-point Likert scale the extent to which each word described the way s/he felt at the current time (1 = “not at all” and 5 = “very strongly”). The sub-scales were collapsed into three separate scales: Negative Emotion: (sub-scales sadness and anger); Anxiety and Arousal: (sub-scales anxiety and fear); Positive Mood: (sub-scale joy and relaxed state).

Cognitive Measure: Stroop Color/Word Test (Golden, 1976): The Stroop test has been used extensively in both clinical and experimental fields to assess the ability to inhibit incongruent competing conflicts. In the interference trial 100 words are presented, and in all cases, the word (i.e. red) is different from the color it is printed in (i.e. blue. (e.g. “red”). Subjects are given 45 seconds to read aloud the color of the ink as quickly as possible. The number of raw items read for each trial is recorded and converted to standardized T scores.

Cardiovascular Measures: A Critikon Dinamap 120 Patient Monitor (GE Medical Systems, Tampa, FL) was used to assess blood pressure and a pulse sensor was attached to the subject's finger to provide a continuous measure of pulse.

2.2. Blood Samples

Cortisol Extraction and Analysis: Cortisol samples were obtained and analyzed as described in Fox et al. (2013). In brief, cortisol samples were obtained in heparinized plasma collection tubes and aliquoted after being centrifuged at 4C within 30 minutes of collection and subsequently stored at -70C. All plasma samples were processed at the Yale Center for Clinical Investigation Core Laboratories using standard radioimmuno-assay procedures. Blood samples for progesterone levels were obtained in serum collection tubes and kept at room temperature for two hours prior to being centrifuged. All samples were then shipped to Graham Massey Analytical Lab, Inc (Shelton, CT) for standard immune-assay procedures.

Neuroactive Steroid Extraction and Analysis: Study day 5 (pre-imagery) plasma levels of the progesterone metabolite (3 α ,5 α)-3-hydroxypregnan-20-one (allopregnanolone) and the

testosterone metabolite 3 α ,5 α -androstenediol (androstenediol) were measured using gas chromatography/ mass spectrometry (GC/MS) with electron capture- negative chemical ionization as previously described (Milivojevic and Covault, 2013; Milivojevic et al., 2014a).

Steroid extraction and separation: In brief, 0.3 ml of plasma, in parallel with a 6-level dilution series of neuroactive steroids standards (Steraloids, Newport, RI) diluted in 5% charcoal-stripped BSA (Sigma Aldrich, St. Louis, MO) were combined with 4 μ l of an internal standard solution containing D4-allopregnanolone (24 pg/ μ l) and D3-androstenediol (16 pg/ μ l) (Cambridge Isotope Laboratories, Andover, MA) to control for variation in sample-to-sample extraction and derivatization efficiencies. 500 μ l of 75% methanol/water (v/v) was added to each sample, and vortex mixed. Samples were diluted with de-ionized water to a final concentration of 5% methanol and then applied to 200 mg C18 solid-phase columns (Varian Corp. Palo Alto, CA; previously conditioned with 6 ml of 100% methanol followed by 6 ml of 5% methanol). The columns were washed with 5% methanol thrice (9 ml total) and steroids eluted with 2 ml of 100% methanol into silinized tubes and dried in a rotary evaporator for approximately 2 hr.

Derivatization: Briefly, samples were reacted as follows: 50 μ l toluene was added to each sample, vortex mixed, and allowed to sit at room temperature (RT) for 5–10 min to solubilize the dried sample; 25 μ l of 40% pyridine (pyridine/toluene; 40/60 v/v) was then added, vortex mixed, and allowed to sit at RT for 3 min; and was followed by the addition of 25 μ l of 10% pentafluorobenzoyl chloride in toluene (PFBC; Sigma–Aldrich, St. Louis, MO), vortex mixed, and incubated for 10–12 min at room temperature. In batches of 8, the samples were then mixed with 0.6 ml of de-ionized water and vortex mixed three times for 10 sec each. The steroids were then extracted twice using 1 ml of hexane and vortex mixing for 20 sec for each extraction.. The pooled hexane extract samples were then dried in a rotary evaporator for approximately 2 hr. All solvents were anhydrous of analytical grade (Sigma-Aldrich; St. Louis, MO).

Instrumentation: The analysis was performed using a Hewlett-Packard (HP) 5890 gas chromatograph coupled to a HP 5988B mass spectrometer using select ion monitoring mode for primary m/z peaks for compounds of interest to increase sensitivity. A thin-film capillary column (30 m $\times$ 0.25 mm, 0.1 μ m film thickness; Restek, Bellefonte, PA) was used to resolve the hydroxyl-reacted pentafluorobenzoyl steroid derivatives. The ratio of the molecular ion peak for each steroid relative to the corresponding area of the density labeled internal standard was calculated for each sample, and using a standard curve generated with each run, the ratio was converted to steroid concentrations. Each sample was run in duplicate, and a sample average was used for data analysis.

2.3. Data and Statistical Analysis

All statistical analyses were performed using SPSS software (SPSS Inc., Version 19, Chicago, IL, USA). Figures were created with GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA). As a manipulation check, ALLO levels during the progesterone/ placebo drug regimen and also pearson's correlation and linear regression analyses were

used to examine correlation between progesterone and ALLO levels. ALLO groupings were generated using a median split. ALLO group differences on stress response, drug craving and inhibitory control were assessed using Linear Mixed Model Effects. Within-subject factors of imagery condition, time point and sex and the between-subjects factor of ALLO group was the fixed effect. Subjects were the random effect. Independent samples t-test and chi square analyses were used to compare demographic characteristics of the subjects in the placebo and the progesterone group. Cortisol data was log transformed to address variability across subjects.

3. Results

3.1. Allopregnanolone and Androstenediol Levels

As expected, levels of the neuroactive progesterone metabolite allopregnanolone (ALLO) were significantly higher ($F(1,42) = 22.4$; $p < 0.001$) in the progesterone compared to the placebo arm in both cocaine dependent men and women. Progesterone levels positively correlated with ALLO levels ($r(39) = .71$; $p < 0.001$), and regression analysis revealed that levels of progesterone significantly predicted levels of ALLO ($R^2 = .51$, $F(1,39) = 39.83$; $p < 0.001$). Sex was included in the analysis and had no effect on the correlation. Progesterone had no effect on levels of the testosterone metabolite androstenediol (ADIOL). ALLO Grouping: In order to examine the effects of progesterone derived baseline ALLO levels on stress, drug craving and inhibitory control outcomes before imagery (pre-imagery baseline) and after imagery (imagery-related change from baseline), subjects were grouped using a median split into a Low ALLO ($n = 22$) and a High ALLO ($n = 24$) group. All results that follow are presented as outcomes of this grouping.

3.2. Sample

The low vs. high ALLO groups did not significantly differ on any demographic characteristics (Table 1). The groups did not differ in sex or race distribution. Participants in both groups were similar in age and years spent in education. The majority of participants were daily smokers, however both groups had a similar number of regular daily smokers and there were no significant group differences on smoking status. There was no difference in years of cocaine use and number of days of cocaine use in the past month between the groups. Similarly the groups did not differ in years of alcohol use or the number of days of alcohol use in the past month. Importantly, whereas a considerable proportion of participants met lifetime criteria for alcohol dependence, there were no significant group differences in this measure. Lastly, participants in both groups did not differ in their prevalence of lifetime depression.

The average ALLO concentration was 101.14 pg/ml (± 16.8) in the Low ALLO group and 8846.79 pg/ml (± 1747.15) in the High ALLO group (Fig 2a). There were no main effects of sex as both cocaine dependent men and women in each group had similar levels of ALLO (Fig. 2b). There were no differences in ADIOL levels in the Low ALLO ($217.2 \text{ pg/ml} \pm 30$) and High ALLO group ($218 \text{ pg/ml} \pm 29$) (Fig. 2c), however ADIOL levels were significantly higher in cocaine dependent men compared to cocaine dependent women ($F(1,42) = 75.5$; $p < 0.001$) (Fig. 2d).

Effects of ALLO on Mood, Stress Response, Drug Craving and Stroop Performance

PRE-IMAGERY BASELINE

BEHAVIORAL MEASURES:

3.3. Positive Emotion (DES)

The high ALLO group reported higher baseline positive emotion ($F(1,43)=10.89$; $p=0.002$) compared to the low ALLO group across all three days (Fig. 3a). No main effect or interaction with sex was observed. There were no significant effects of ALLO group on pre-imagery craving or Stroop task performance.

PHYSIOLOGICAL MEASURES:

3.4. Cortisol

There was an overall main effect of ALLO on pre-imagery levels of cortisol ($F(1,44)=3.93$; $p=0.05$), such that the high ALLO group had lower baseline levels of cortisol across all three imagery days compared to individuals with low levels of ALLO (Fig. 3b). Again, we didn't observe a main effect of sex or a sex x ALLO group interaction on pre-imagery baseline levels of cortisol. There was no difference between ALLO groups for pre-imagery SBP.

IMAGERY-RELATED CHANGE FROM BASELINE

BEHAVIORAL MEASURES:

3.5. Stroop Performance

An ALLO x Condition interaction ($F(2,132)=3.5$; $p=0.03$) indicated that cocaine dependent individuals with high levels of ALLO demonstrated higher Color/Word scores post- imagery time-points relative to pre-imagery baseline assessment compared to individuals with low levels of ALLO, specifically in response to the cue and stress conditions (Fig. 4a). No main effect or interaction with sex on Stroop performance was observed.

3.6. Cocaine Craving

A main effect of ALLO ($F(1,41)=3.99$; $p=0.05$) indicated that the high ALLO group had significantly lower post-imagery cocaine craving across all three conditions compared to individuals that had low levels of ALLO (Fig.4b). Again, there was no main effect or interaction with sex on cocaine craving. There was no significant effect of ALLO group on post-imagery positive emotion.

PHYSIOLOGICAL MEASURES:

3.7. Cortisol Levels

An ALLO x Condition effect showed that the high ALLO group had a greater change in cortisol in the stress condition across all time points as compared with individuals with low levels of ALLO ($F(2,64)=3.15$; $p<0.05$) (Fig. 5a). No main effect or interaction with sex was observed and there were no effects of the other imagery conditions on cortisol levels.

3.8. Systolic Blood Pressure (SBP)

An ALLO x Condition effect indicated that cocaine dependent individuals in the high ALLO group had a smaller change in SBP ($F(2,195)=2.72$; $p=0.02$) in response to drug cue across all time points compared to individuals in the Low ALLO group (Fig. 5b) in both males and females. There were no interaction effects of the other imagery conditions.

4. Discussion

The present study examined the effects of the progesterone derived neuroactive steroid allopregnanolone levels on stress response, drug craving and inhibitory control in a sample of cocaine dependent men and women who received progesterone or placebo for seven days in early abstinence. As expected, we found ALLO levels to be highly correlated with progesterone administration and that cocaine dependent individuals administered progesterone showed significantly higher ALLO plasma levels relative to the placebo treated group. However, the levels of the testosterone derived neuroactive steroid androstenediol were unaffected by progesterone-derived ALLO levels or by progesterone administration. In assessing the effects of high versus low ALLO levels on mood, stress, drug craving and Stroop performance, we found that the High versus Low ALLO group showed reduced blood pressure, and better cognitive inhibitory control performance as measured by the Stroop task in response to stress and cocaine cue, higher positive emotion at baseline and overall lower peak cocaine craving. Increased plasma levels of ALLO were associated with reduced cortisol levels at pre-imagery and a normalized cortisol response to stress. There were no sex specific effects of ALLO on these responses. These findings are consistent with previous observations in our laboratory that progesterone has inhibitory effects on stress responsivity and drug craving in cocaine dependent men and women (Sinha et al., 2007; Fox et al., 2013), and further extend the current findings to suggest that the progesterone metabolite ALLO, which is a known neuroactive steroid, may play a significant role in accounting for a number of the progesterone effects on mood, drug craving, stress response and inhibitory control in cocaine dependence. In addition, it appears that unlike progesterone's sex-specific effects on negative affect, positive emotion and blood pressure (Fox et al., 2013), ALLO's effects were not sex-specific, with similar effects in men and women, pointing to a possibly ALLO-specific mechanism, where women might be more sensitized to the effects of progesterone but not its derivative ALLO.

The findings suggest that future research may benefit from developing strategies to increase endogenous ALLO and assess its potential therapeutic benefit in the treatment of cocaine dependence. In the current study, progesterone selectively increased levels of its metabolite ALLO and had no effects on the testosterone metabolite ADIOL. Moreover, administration of progesterone had the same effect on ALLO levels in both men and women. All women in this study were in their follicular menstrual cycle phase at the time of progesterone administration and assessments, and thus their endogenous levels of ALLO were low and similar to those of men. As expected, levels of the testosterone derived ADIOL were higher in men than women.

With regard to mood, we observed improvements in positive emotion in those individuals that had high levels of ALLO. This is consistent with other studies that have found gonadal

hormones to affect emotion regulation as well as affective states (for review refer to van Wingen et al. (2011)). For example, fluctuations in endogenous progesterone levels during the menstrual cycle have revealed that low progesterone levels are associated with basal negative mood in healthy women (Backstrom et al., 1983; Sveindottir and Backstrom, 2000) and increased anxiety in response to stress in cocaine-dependent women (Sinha et al., 2007). It is possible that these processes are modulated by sex hormone derived neuroactive steroids, although further research needs to assess the role of allopregnanolone in emotion regulation more directly.

High levels of ALLO were also associated with a blunted SBP response to cocaine cues which have been shown to be a variant stressor associated with high levels of arousal and appetitive, anxiety-type negative mood (Fox et al., 2007; Fox et al., 2008a). Again, ALLO's inhibitory effects on central nervous system projections could contribute to this attenuated cardiovascular response to cue, or findings could reflect an indirect effect of increased relaxed state and positive mood at baseline.

The current findings also demonstrated that high levels of ALLO were associated with lower levels of cortisol at pre-imagery, but a higher cortisol response to stress imagery; in other words, we observed a less blunted cortisol response to stress in cocaine dependent individuals with high levels of ALLO. This is important, because cocaine dependent individuals in early abstinence demonstrate a tonic upregulation in baseline cortisol levels (Fox et al., 2008a; Fox et al., 2009), and a blunted reactivity to stress (Back et al., 2010). Thus, consistent with this previous literature, current findings suggest that high ALLO levels may normalize the high basal cortisol and blunted HPA response to stress in cocaine dependence. This normalization of the HPA axis response in stress with high levels of ALLO may have significant clinical implications in chronic cocaine abuse.

In this study, high levels of ALLO were associated with an improved Stroop performance, a task known to measure inhibitory control and cognitive flexibility. Early abstinence from cocaine is associated with robust GABAergic and corticotropin-releasing factor (CRF) adaptations within selective regions of the prefrontal cortex that are concomitant with cognitive and inhibitory control dysregulation (George et al., 2012; Peters et al., 2008). Allopregnanolone increases GABAergic currents and thereby modulates GABA-activated inhibition of neural firing in dopaminergic regions of the prefrontal cortex (Becker, 1999; Becker and Hu, 2008; Russo et al., 2008). Such increases in GABA-activated neural inhibition may represent a possible mechanism to explain current findings of overall improved Stroop performance in cocaine dependent individuals treated with progesterone. Overall improvements in inhibitory control in addicts could provide significant clinical value, as impairments in inhibitory control and enhanced impulsivity are strong risk factors for the development of dependence and high relapse rates (Czapla et al., 2015). This finding is consistent with previous research demonstrating that endogenous and exogenous increases in progesterone improved cognitive deficits in cocaine dependence (Milivojevic et al., 2014b), and current findings are further proof that these effects may be mediated by progesterone derived neuroactive steroids.

Finally, the current study showed that the high ALLO group showed decreased cocaine craving in response to both cue and stress, and also in the neutral condition relative to the low ALLO group. The effects of neuroactive steroids on cocaine craving have not been previously assessed, however this finding is consistent with studies from our laboratory which found that high levels of endogenous (Sinha et al., 2007) and exogenous (Fox et al., 2013) progesterone reduced cue and stress induced cocaine craving.

The interpretation of the findings in the current study has some limitations. One limitation is the study sample, which consisted of cocaine dependent individuals that also abused alcohol and were regular daily smokers. Although this may represent an ecologically valid cocaine sample as many experimental and clinical studies have indicated that the simultaneous abuse of alcohol and nicotine is typical in cocaine dependence (McCance-Katz et al., 1999; Patkar et al., 2006), it does not allow us to extrapolate the findings to a “pure” cocaine dependent sample. Importantly, since previous studies have shown that chronic alcohol exposure reduces allopregnanolone levels, the current results might suggest that concurrent alcohol abuse would further add to the detrimental symptoms of cocaine dependence. Another limitation is that we only measured levels of allopregnanolone at one time point on day 5 of progesterone treatment, and future research should assess the role of phasic levels of allopregnanolone in cocaine dependence, such as those in response to stress and cocaine cue provocation. Similarly, cortisol varies across the day and we saw the effect for morning fasting cortisol under basal and provocation conditions, however we do not know whether ALLO affects the diurnal rhythm and/or cortisol levels during other periods of the day. To our knowledge, this is one of the first studies to examine progesterone-derived neuroactive steroid, allopregnanolone, and assess its effects on acute stress response and drug craving in a sample of cocaine dependent men and women. Despite the study limitations, the findings indicate that high ALLO levels may have beneficial effects on mood, inhibitory control, stress arousal and cocaine craving. Importantly, these effects were observed irrespective of sex, as both cocaine dependent men and women showed similar increases in ALLO with the same dose of progesterone, and experienced similar benefits. As these findings demonstrate that progesterone induced increases in ALLO levels may play a role in improving early abstinence symptoms in cocaine dependent individuals, further research is warranted to elucidate the more specific role that neuroactive steroids may play in cocaine dependence.

Acknowledgements

This research was supported by grants P50-DA016556 (Dr. Sinha), R01-AA20095 (Dr. Fox), 5T32DA007238-24 (Dr. Milivojevic), P60 AA03510 (Dr. Covault; UConn Alcohol Research Center) from the National Institutes of Health and the State of Connecticut. We would also like to thank Richard Lieberman, the staff at The Clinical Neuroscience Research Unit, The Substance Abuse Center at The Connecticut Mental Health Center and the Yale Center for Clinical Investigation (YCCI) supported by the Yale Clinical and Translational Science Award (Yale CTSA, UL1-RR024139) for their assistance in completing this study.

Funding Source This research was supported by grants P50-DA016556 (Dr. Sinha), R01-AA20095 (Dr. Fox), 5T32DA007238-24 (Dr. Milivojevic), P60 AA03510 (Dr. Covault; UConn Alcohol Research Center) from the National Institutes of Health and the State of Connecticut. We would also like to thank Richard Lieberman, the staff at The Clinical Neuroscience Research Unit, The Substance Abuse Center at The Connecticut Mental Health Center and the Yale Center for Clinical Investigation (YCCI) supported by the Yale Clinical and Translational Science Award (Yale CTSA, UL1-RR024139) for their assistance in completing this study.

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Highlights

Progesterone was used to increase allopregnanolone (ALLO) levels in cocaine addicts.

Individuals with High ALLO levels demonstrated a normalized HPA axis function.

Individuals with High ALLO levels displayed increased cognitive performance.

Individuals with High ALLO levels experienced reduced cocaine craving.

Increasing allopregnanolone levels in CD individuals may provide therapeutic benefit.

Figure 1.

Schematic illustration of the laboratory session timeline. Cocaine craving, subjective emotion, Systolic Blood Pressure (SBP) and cortisol were collected at all time points after the Set up and Adaptation Period (Pre-Imagery Baseline, Imagery, R1, R2, R3, R4, R5). Stroop performance was assessed at the Pre-Imagery Baseline and the Imagery time points only. ALLO and ADIOL levels were measured at the Pre-Imagery Baseline time point only.

* No measures were taken during the Set up and Adaptation Period time point.

Figure 2.

(A) Plasma basal levels of allopregnanolone as a function of ALLO grouping. Data was collapsed for men and women, as there was no significant sex effect. (B) Plasma basal levels of allopregnanolone as a function of ALLO grouping in men and women, demonstrating lack of sex differences. (C) Plasma basal levels of androstenediol as a function of ALLO grouping; (D) androstenediol levels as a function of sex. Levels of the testosterone derived androstenediol did not differ in the Low versus High ALLO groups, but levels were higher in men than women. Data are displayed as mean $\pm$ S.E.M. *** $p < 0.001$.

Figure 3.

(A) Positive Emotion Score as a function of allopregnanolone (ALLO) group at pre-imagery baseline. Higher levels of positive emotion at baseline were found in the High versus the Low ALLO group across all three imagery days. (B) Log-transformed cortisol levels as a function of allopregnanolone (ALLO) group at pre-imagery baseline across all three imagery days. Data are displayed as mean $\pm$ S.E.M. * $p < 0.05$; ** $p < 0.01$.

Figure 4.

(A) Stroop Performance post-imagery relative to pre-imagery baseline as a function of allopregnanolone (ALLO) group. ALLO group X Condition effects showed overall higher Stroop performance in response to stress and cue in the high ALLO relative to low ALLO group. (B) Change in Craving Score as a function of allopregnanolone (ALLO) group. Craving, averaged across all time points, was lower in all three conditions in the high ALLO group as compared to the low ALLO group. All data are displayed as mean $\pm$ S.E.M. * $p < 0.05$.

Figure 5.

(A) Log-transformed data of ALLO group X Condition effects in cortisol response averaged across all post-imagery time points showed a higher cortisol response to stress in the high ALLO relative to low ALLO group. (B) Change in Systolic Blood Pressure (SBP) averaged across all time points. ALLO group X Condition effects showed overall lower SBP in response to cue in the high ALLO relative to low ALLO group. Data are displayed as mean $\pm$ S.E.M. Data are displayed as mean $\pm$ S.E.M. * $p < 0.05$.

Table 1

Demographic Characteristics of Sample.

	Low ALLO n=22	High ALLO n=24
Sex – no. of males	13	16
Race		
African American	15	19
Caucasian	6	4
Other	1	1
Age (s.d.)	40.77 (8.2)	42.17 (5.6)
Years spent in education (s.d.)	12.07 (1.75)	12.17 (1.47)
No. of regular smokers	19	23
Years of cocaine use (s.d.)	13.86 (8.7)	12.65 (5.7)
No. of days used in past month (s.d.)	13.64 (8.9)	14.39 (8.4)
Years of alcohol use (s.d.)	13.14 (8.4)	16.27 (10.3)
No. of days used in past month (s.d.)	9.36 (9.3)	11.39 (9.5)
Lifetime alcohol dependence	16	18
Lifetime depression	1	1