HISTORIES OF DEPRESSION, ALLOPREGNANOLONE RESPONSES TO STRESS, AND PREMENSTRUAL SYMPTOMS IN WOMEN

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ABSTRACT

REBECCA R. KLATZKIN: Histories of Depression, Allopregnanolone Responses to Stress, and Premenstrual Symptoms in Women
(Under the direction of Susan S. Girdler)

Twenty-six women with premenstrual dysphoric disorder (PMDD) and 39 non-PMDD women were tested for allopregnanolone (ALLO) responses to mental stress. Fourteen PMDD and 17 non-PMDD women had a history of depression (DEP), though all were free of current psychiatric illness. Women with prior DEP showed a blunted ALLO stress response and failed to show a decrease from venipuncture to baseline compared to women with no prior DEP. Women with prior DEP did not show the correlation between progesterone and ALLO that was seen in those with no prior DEP. ALLO levels at baseline and blunted ALLO reactivity predicted more severe premenstrual symptoms, but only in PMDD women with prior DEP. These results suggest that prior DEP is associated with a failure of ALLO to be appropriately responsive to challenge, with alterations in the conversion of progesterone to ALLO, and link ALLO to symptoms in PMDD women with prior DEP.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Table Page

LIST OF FIGURES

LIST OF ABBREVIATIONS

ALLO	Allopregnanolone
DEP	History of Depression (includes the diagnoses of prior major depressive disorder, minor depressive disorder, or adjustment disorder with depressed mood)
PMDD	Premenstrual Dysphoric Disorder
PRISM	Prospective Record of the Impact and Severity of Menstrual Symptoms
TSST	Trier Social Stress Test

CHAPTER 1
INTRODUCTION

Premenstrual dysphoric disorder (PMDD) is estimated to afflict 5–8% of women in their reproductive years (American Psychiatric Association, 1994). PMDD is characterized by premenstrual emotional and physical symptoms severe enough to interfere with function during the last week of the luteal phase of the menstrual cycle, but which remit with the onset of menses. Despite more than 60 years of research into this disorder, the underlying pathophysiologic mechanisms remain elusive. Owing to the cyclical nature of the mood changes in PMDD, early research focused on the gonadal steroid hormones (Dalton, 1964). However, despite the overwhelming evidence for an obligatory role of the gonadal hormones in the pathophysiology of PMDD, it is generally agreed that neither a deficiency nor excess in progesterone or estradiol is etiologically relevant to the disorder (Rubinow et al., 1988).

Although differences in absolute levels of gonadal hormones may not be of clinical relevance in PMDD, women with this disorder may be more sensitive to the mood modulatory effects of gonadal hormones (Hammarback et al., 1989), or there may be alterations in the conversion of these hormones to their neuroactive metabolites and a differential sensitivity to these metabolites in PMDD. Of particular relevance to PMDD may be the neuroactive steroid allopregnanolone (3α-hydroxy-5α-pregnan-20-one). Allopregnanolone (ALLO) is the A-ring-reduced metabolite of progesterone and is produced not only by ovary and adrenals but also de novo in brain (Paul and Purdy, 1992). Owing to its lipophilicity, even peripherally produced ALLO readily crosses the blood brain barrier

where it rapidly alters CNS excitability, producing behavioral effects within seconds to minutes (Paul and Purdy, 1992). ALLO is a potent modulator of $GABA_A$ receptors (nanomolar concentrations) via dose-dependent enhancement of GABA-induced Cl^- ion channels (Morrow et al., 1987), and it is through this mechanism that it exerts profound anxiolytic effects (Brot et al., 1997).

There is a small but growing literature on neurosteroids in prospectively diagnosed PMDD women. These studies have yielded mixed results, however, finding either no diagnosis-related difference in ALLO levels (Epperson et al., 2002; Schmidt et al., 1994; Wang et al., 1996), or significantly lower ALLO levels in PMDD women compared with controls (Lombardi et al., 2004, Monteleone et al., 2000; Rapkin et al., 1997). There are several methodological issues that could contribute to the discrepant findings, including differences in the diagnostic criteria employed, since some (Rapkin et al., 1997; Wang et al., 1996), but not all (Epperson et al., 2002; Lombardi et al., 2004; Monteleone et al., 2000; Schmidt et al., 1994) of these studies used strict Diagnostic and Statistical Manual of Mental Disorders (DSM; American Psychiatric Association, 1994) criteria for PMDD. Additionally, in the prior studies, either psychiatric histories were not assessed (Monteleone et al., 2000; Wang et al., 1996), they were assessed in the PMDD women but not controls (Schmidt et al., 1994), or women with recent psychiatric histories were excluded (Rapkin et al., 1997; Lombardi et al., 2004). Since reduced ALLO levels have been documented in major depression (Nappi et al., 2001; Strohle et al., 2000; Strohle et al., 1999; Romeo et al., 1998; Uzunova et al., 1998), and because a history of major depression may be more common in PMDD (Pearlstein et al., 1990), study-related differences in lifetime psychiatric comorbidity could contribute to the mixed results.

In contrast to these other reports, our earlier study (Girdler et al., 2001), used strict DSM criteria to classify PMDD, and also used structured clinical interview to exclude both PMDD and non-PMDD women with current psychopathology. With this careful approach, we found that PMDD women had greater luteal phase concentrations of ALLO than non-PMDD women. The initial study was limited, however, by the small number of non-PMDD controls versus PMDD patients (n=13 vs. 25), and by our failure to examine whether the groups differed in lifetime histories of depression. Despite the discrepancies in the literature on ALLO in PMDD, the results of the existing studies, taken together, still raise the possibility that ALLO plays a role in PMDD since our prior study (Girdler et al., 2001) and the study of Wang et al. (1996) found a relationship between ALLO concentrations and symptom severity in PMDD women.

Given the role of stress in the etiology and exacerbation of psychiatric symptoms (Kendler et al., 2004), combined with the ability of ALLO to modulate mood via $GABA_A$ receptors, dysregulation in the ALLO response to stress represents a potential mechanism by which ALLO may have pathophysiological significance. ALLO has been shown to be stress sensitive in rat models, with CNS levels rising quickly following acute stress (Barbaccia et al., 1996; Purdy et al., 1991). Peripheral levels in rat also increase significantly following stress, though the response is more delayed (Paul and Purdy, 1992). Animal models indicate that stress-induced increases in ALLO serve to negatively modulate hypothalamic-pituitary-adrenal (HPA) axis activity, thereby facilitating the recovery of physiologic homeostasis in this system following stressful stimuli (Guo et al., 1995; Patchev et al., 1996). In humans, Genazanni et al. (1998) employed endocrine challenge paradigms in healthy men and women, and found that both GnRH and CRH administration increased serum ALLO levels

whereas suppression of adrenal steroidogenesis markedly reduced ALLO. These early results suggested that in humans, both the ovaries and the adrenal cortex are major sources of circulating ALLO, and that neuroactive steroids may be stress sensitive in humans as well.

Our prior study (Girdler et al., 2001) was the first to examine ALLO responses to mental stress in human females. We assessed ALLO after an extended baseline rest and again 17 minutes after the onset of mental stressors. Only non-PMDD controls showed the expected stress-induced increase in ALLO, while PMDD women showed a blunted ALLO response. Our initial study did not, however, examine ALLO responses to stress as a function of psychiatric histories. Since lifetime psychiatric illness may be more prevalent in PMDD populations (Pearlstein et al., 1990), one explanation for the blunted ALLO response to stress in PMDD women may relate to their greater likelihood of having lifetime depression.

Consequently, the first objective of the present study was to extend our prior work by examining whether histories of depression in women are associated with alterations in ALLO responses to stress. Second, we sought to provide an initial assessment of the time course of the ALLO response to stress in humans by sampling ALLO at both 30 minutes and 60 minutes post-stress onset, since these are time points associated with the peak cortisol response to mental stress in humans (i.e., 30 minutes) (Kirschbaum et al., 1995a; Kirschbaum et al., 1995b) and the peak plasma ALLO response to stress in animals (i.e., 60 minutes) (Purdy et al., 1991). Third, we investigated whether ALLO concentrations and reactivity to stress predicted premenstrual symptoms in PMDD women.

CHAPTER 2
METHODS

Subjects

Twenty-six women meeting DSM IV criteria for PMDD and 39 controls, who responded to newspaper, radio, or posted advertisements, served as subjects. Based on the evidence for an association of PMDD with lifetime depression (Pearlstein et al., 1990) we did not engage in selective recruitment efforts for PMDD women with histories of depression. To recruit non-PMDD controls with prior depression, a proportion of our advertisements targeted women with histories of depression. All subjects were in good health, reported regular menstrual cycles, and were free of any current psychiatric Axis I disorder, as determined by structured interview (see below). No participant was taking any prescription medication, including oral contraceptives or psychotropic agents. The protocol was approved by the University of North Carolina at Chapel Hill, Committee on Protection of the Rights of Humans Subjects. Subjects provided written informed consent before participation and each received a minimum of $500 compensation upon completion of the study.

Assessment of PMDD

The Prospective Record of the Impact and Severity of Menstrual Symptoms (PRISM) calendar (Reid, 1985) was used for the daily quantification of the severity of physical and emotional/behavioral symptoms that occur over the course of a menstrual cycle. For each symptom listed, subjects assigned a 0 if absent, a l if mild (noticeable but not troublesome), a 2 if moderate (interfering with normal activity), or a 3 if severe (temporarily incapacitating).

In addition, the PRISM calendar incorporates measures of lifestyle impact together with information on life events and the use of medications that may modify symptomatology. All subjects completed the calendars on a daily basis for two to three menstrual cycles. To discourage retrospective reporting, subjects mailed back the calendars weekly.

Criteria for PMDD were based upon those of the DSM-IV (American Psychiatric Association, 1994), that include: (1) rating of symptoms as moderate or severe (as opposed to mild) premenstrually; (2) at least two moderate to severe symptoms on at least three of the six premenstrual days; (3) at total of five or more symptoms premenstrually; (4) at least one moderate to severe emotional symptom on at least three of the six premenstrual days; (5) at least 6 days during the follicular phase with no emotional symptoms and no more than mild physical symptoms; and (6) criteria 1–5 met on two consecutive menstrual cycles. Additionally, functional impairment in PMDD women was confirmed by visual inspection of the PRISM calendar to insure that luteal phase symptoms were associated with lifestyle impact (e.g., time off work; increased aggression) that was not evident during symptom-free days. Women classified as non-PMDD controls 1) were not completely asymptomatic during the premenstrual week (to exclude women biased toward nonreporting), 2) had only mild emotional symptoms premenstrually, 3) had moderate physical symptoms on fewer than 3 days premenstrually and no severe physical symptoms, and 4) criteria 1–3 met on two consecutive menstrual cycle. Additionally, visual inspection of the PRISM calendar was used to confirm that the premenstrual symptoms in the non-PMDD women were not associated with lifestyle impact.

Psychiatric and Abuse Histories

Structured clinical interviews (SCID) based on DSM-IV criteria for Axis I disorders were conducted in the follicular phase of the menstrual cycle. All diagnoses were based on a consensus diagnostic session with a senior psychiatrist (CP). In the present study, we defined prior depression (DEP) to include the diagnoses of major depressive disorder, minor depressive disorder, or prior adjustment disorder with depressed mood. Based on these criteria, 14 of the 26 (54%) PMDD women were classified with prior DEP while 17 of the 39 (44%) non-PMDD controls were classified with prior DEP. For past depressive disorders, 7 months in full remission was required before testing. For other Axis I disorders, 3 years in full remission was required.

Since lifetime psychiatric disturbance and histories of abuse are associated (Kendler et al., 2000), at the end of the SCID, sexual and physical abuse histories were assessed using a validated interview (Leserman et al., 1996). Preliminary analyses indicated no independent effects of abuse on ALLO measures. Thus, abuse histories are included in the present report as a potential predictor of ALLO only. As we have previously observed (Girdler et al., 2003), more PMDD women in the present study had histories of sexual and physical abuse than non-PMDD controls (11 vs. 9, $F(1,58) = 3.62$, $p = .06$).

Experimental Procedures

In addition to examining ALLO reactivity to stress, this study had other aims, including examining menstrual cycle effects on cardiovascular stress reactivity (results to be reported elsewhere). Thus, each woman was tested twice, one in the early follicular phase (days 2-6) and once in the luteal phase, 8-12 days after home urine testing revealed the LH surge that precedes ovulation by 24-36 hours. Order of phase at first testing was

counterbalanced within PMDD and non-PMDD groups. Since ALLO is a metabolite of progesterone, and our prior study was unable to detect significant ALLO concentrations in the follicular phase in a substantial proportion of women (Girdler et al., 2001), the present report focuses exclusively on ALLO measures obtained during the luteal phase.

After instrumentation for cardiovascular monitoring, subjects were escorted to a sound-attenuated testing chamber and seated in a comfortable chair. Next, an intravenous (i.v.) line was established in an arm vein and once the i.v. was in place, the first blood sample for ALLO was taken, providing an index of the ALLO stress response to the novel environment and venipuncture. Then, a curtain was drawn that prevented the subject from viewing the i.v. apparatus and blood sampling during the remainder of the procedures.

Extended baseline rest

Immediately following the i.v. setup (approximately 5 minutes), a series of stethoscopic blood pressures was taken (5 minutes) and questionnaires completed (5 minutes). Then, 10 minutes of quiet rest followed. Blood was sampled for extended baseline ALLO at minute 10 of this period (approximately 25 minutes after venipuncture). This blood sample also provided the estradiol and progesterone concentrations in order to confirm menstrual cycle phase.

The Trier Social Stress Test (TSST)

A modified version of the TSST was employed (modified to account for the fact that our subjects were not ambulatory and also modified to include serial addition as opposed to serial subtraction). The TSST is a stress test that reliably induces large and consistent HPA and cardiovascular responses (Kirschbaum et al., 1993; Kirschbaum et al., 1995a; Kirschbaum et al., 1995b). The TSST involves four components: 1) *Pre-Task Instructions*

(5 min) during which time subjects are introduced to the 'selection committee' who will later listen to their job talk. Subjects are also given the instructions for the mental arithmetic task. The duration of the instruction period averaged 5 minutes; 2) *Speech Preparation Period (5 min)*: during which time subjects were left alone for 5 minutes to prepare their talk. 3) *Job Speech (5 min)*: immediately following the preparation period, the selection committee returned to the testing room and asked the subject to deliver her talk describing to the committee why she would be the perfect applicant for the position. If the subject finished before 5 minutes, the committee responded in a standardized way, with prepared questions to ensure that the subject spoke for the entire period. Talks were tape-recorded and subjects had the opportunity to earn up to $10 based on the committee's ratings of their speech performance; and 4) *Paced Auditory Serial Addition Test* (PASAT; Gronwall, 1977) (8.5 min) involves the tape recorded presentation of numbers from 1 – 9. Subjects are to add each number presented on the tape to the immediately preceding number and to state the answer aloud. There are four series of numbers, with progressively shorter inter-digit intervals. The experimenter remained in the room to monitor performance. Subjects had the opportunity to earn up to $10 based upon the total number of correct additions.

Stress Recovery (10 min)

Subjects rested quietly alone following the mental stressors. Blood was sampled at the end of this recovery period (minute 10) since this is the time point when cortisol shows a peak response to the TSST (Kirschbaum et al., 1995a; Kirschbaum et al., 1995b). Thus, this ALLO sample constituted the 30-min post-stress onset sample. The next ALLO sample was taken 30 minutes after the end of recovery, since this is a time point when animal models indicate peak plasma ALLO responses to stress (Purdy et al., 1991). Thus, this sample

provided the 60-minute post-stress onset sample. Subjects rested quietly alone during the 30-minute period that separated these samples.

Allopregnanolone and Progesterone Assays

Plasma ALLO (3α,5α-THP) was assessed by radioimmunoassay following extraction and purification by column chromatography as previously described (Janis et al., 1998; Girdler et al., 2001). The 3α,5α-THP antiserum has previously been shown to produce minimal cross reactivity with other circulating steroids (Janis et al., 1998). Cross-reactivity with progesterone (<3%), as well as the stereochemical isomers of 3α,5α-THP is minimal (3α,5β-THP 6.6%; 3β,5α-THP 2.8%; 3β,5β-THP 0.5%). In contrast, the steroid 3α-hydroxy-4-pregnen-20-one binds to the antibody to a greater degree than 3α,5α-THP (169% of 3α,5α-THP). It is unknown, however, whether 3α-hydroxy-4-pregnen-20-one exists in human serum. If the steroid does exist in human serum, then it may contribute to the measurement of ALLO; however, because both ALLO and the pregnen-4 compound are equally efficacious agonists of $GABA_A$ receptor mediated Cl^--uptake (Morrow et al., 1990), they would be expected to produce similar effects.

Serum progesterone were determined using radioimmunoassay (RIA) kits commercially available from ICN Biomedical, Inc. The specificity of the assay for progesterone is very high, showing only 0.01–2.5% cross-reactivity with other steroid compounds. Luteal phase progesterone levels of less than 3 ng/mL are considered reflective of an anovulatory cycle. Because prior detection of the midcycle LH surge accompanying ovulation was a prerequisite for scheduling luteal testing, no subject was tested during an anovulatory cycle. Upon collection, samples for the ALLO assay were immediately cold-centrifuged, vortexed, and the plasma pipetted and frozen at −80°C until the time of assay.

Serum samples for progesterone were allowed to clot at room temperature for 30 min before centrifuging and pipetting.

Data Analysis

Group differences in demographic factors and psychiatric histories were examined using a 2 (Group: PMDD vs. non-PMDD) x 2 (History of DEP: yes or no) analysis of variance (ANOVA) or chi-square analyses. Next, ALLO reactivity to stress was assessed in 2 ways. First, a delta score was calculated separately for the samples taken at 30 and 60 minutes post-stress onset (post-stress level – extended baseline rest level). These delta scores were analyzed using a 2 (Group) x 2 (History of DEP) repeated measures ANOVA, with time point (30 vs. 60 minutes) as the repeated factor. Second, to examine recovery from venipuncture stress, ALLO levels at venipuncture stress and extended baseline rest were compared using a 2 (Group) x 2 (DEP) repeated measures ANOVA with time point (i.v. insertion and extended baseline) as the repeated factor. Next, we examined the relationship of ALLO to progesterone using Pearson product–moment correlational analysis relating extended baseline ALLO levels to progesterone levels.

Our final analytic goal involved examining the effect of prior DEP on premenstrual symptoms, and whether ALLO predicted symptoms in PMDD women. Thus, differences in luteal phase premenstrual symptoms were analyzed using a 2 (Group) x 2 (DEP) ANOVA. In order to minimize Type I error rates, we selected only 8 premenstrual symptoms representing core emotional (depression, anxiety, irritability, anger, labile mood) and physical (cramping, fatigue, headache) symptoms. Multiple stepwise regressions were then performed in order to examine the degree to which selected variables (histories of abuse,

luteal ALLO at extended baseline, delta ALLO at 30 min post-stress, and delta ALLO at 60 min post-stress) served as independent predictors of the severity of each premenstrual symptom (based on the mean prospective symptom severity score from the PRISM calendars). Based on our finding that women with prior DEP had more severe premenstrual symptoms (see Results), in order to reduce Type I error rates, we limited our regression analyses to only those symptoms that differed by DEP (i.e. depression, irritability, labile mood, and fatigue). We further limited our regression analyses to PMDD women only, since non-PMDD controls were specifically recruited to have no more than mild symptoms.

Type III sum of squares (SS) was employed for testing all group differences using ANOVA, since we had unequal cell sizes and Type III SS is equivalent to testing the unweighted marginal averages against one another. Of the total 65 women who completed the study, 2 women (1 PMDD and 1 non-PMDD) were missing 30 or 60 minutes post-stress ALLO samples and therefore were not included in the delta ALLO analyses. In addition, certain psychiatric history information (prior anxiety disorders, substance abuse, post traumatic stress disorder (PTSD), and eating disorders) was unavailable for 3 subjects (1 PMDD and 2 non-PMDD), though depression history was available for all participants. Therefore, the chi-square analyses used to examine group differences in prior Axis I disorders is based on 25 PMDD and 37 non-PMDD women (Table 1).

CHAPTER 3
RESULTS

Demographic and Psychiatric Histories

There were no significant differences as a function of group or history of DEP for age, luteal phase progesterone concentration, or percent minorities, though PMDD women with prior DEP had greater BMI's than PMDD women with no prior DEP (Group x DEP: $F(1, 64) = 4.22$, $p<.05$).

As expected, women with histories of DEP were more likely to have had other prior psychiatric illness. Chi square analyses indicated that more PMDD women with prior DEP also had prior eating disorders ($X^2(1) = 4.9$, $p<.05$), while more non-PMDD women with prior DEP also had prior anxiety disorders ($X^2(1) = 6.8$, $p<.01$) and substance abuse or dependence ($X^2(1) = 4.0$, $p<.05$).

Histories of Depression and Allopregnanolone Reactivity to Stress

Women with histories of DEP, regardless of PMDD vs. control status, showed alterations in their ALLO response to stress. As illustrated in Figure 1, women with prior DEP displayed a blunted ALLO stress response at both 30 and 60 minutes post-stress, relative to women with no prior DEP ($F(1,59) = 4.1$, $p<.05$), since their absolute ALLO concentrations were lower at 30 minutes (2.46 ng/mL) and at 60 minutes (2.44 ng/mL) post-stress relative to their concentration at extended baseline (2.67 ng/mL). Women with no prior DEP showed a small increase in ALLO concentration at 30 minutes (2.42 ng/mL) and 60 minutes (2.45 ng/mL) post-stress relative to extended baseline (2.40 ng/mL). Subsequent

examination of the magnitude of the response within groups using paired comparison t-tests indicated that the decrease in ALLO at both 30 and 60 minutes post-stress was significant in the women with prior DEP (ts = -2.09 and –2.37, Ps<.05) while the increase in ALLO at 30 and 60 minutes post-stress in women with no prior DEP was non-significant. In addition, all women with histories of DEP tended to show a lack of recovery from i.v. venipuncture stress (Figure 2). Specifically, ALLO levels in women with prior DEP did not show the expected decrease from venipuncture to extended baseline rest that occurred approximately 25 minutes after venipuncture, that was evident in women with no prior DEP (Time x DEP: F (1,61) = 3.2, p = .08).

Histories of Depression and the Relationship of Allopregnanolone to Progesterone

Only women with no history of DEP, regardless of PMDD vs. control status, showed the expected relationship of ALLO to progesterone. Specifically, women with no prior DEP showed a positive correlation between luteal ALLO levels and luteal progesterone levels (r = .37, p<.05) while no significant relationship was found in women with prior DEP (r = .16, p = NS).

Histories of Depression and Premenstrual Symptoms

As expected, for all 8 premenstrual symptoms, all PMDD women, regardless of DEP histories, exhibited significantly greater symptom severity during the luteal phase than non-PMDD women (F values (1,61) = 12.1 – 341.1, P values<.001). However, history of DEP, regardless of PMDD status, was also associated with greater luteal symptom severity for certain symptoms. Specifically, women with prior DEP reported greater premenstrual depression (F (1,61) = 5.9, p<.05), irritability (F (1,61) = 7.9, p<.01), labile mood (F (1,61) = 3.8, p = .05), and fatigue (F (1,61) = 3.3, p = .07), compared to women with no prior DEP.

There was no influence of prior DEP on severity of premenstrual anger, anxiety, headaches, or menstrual cramps.

Predictors of Premenstrual Symptoms in PMDD Women with Histories of Depression

Table 2 summarizes the multiple stepwise regressions relating selected predictor variables (histories of abuse, luteal ALLO at extended baseline, delta ALLO at 30 minutes post-stress, and delta ALLO at 60 minutes post-stress) to the premenstrual symptoms that were significantly greater in PMDD women with prior DEP (i.e., depression, irritability, labile mood, and fatigue). Although the greater ALLO levels at extended baseline rest most robustly predicted premenstrual symptoms of depression, irritability, and labile mood (R^2 = .30 - .37), blunted delta ALLO at 30 minutes post-stress also independently predicted greater premenstrual depression ratings (R^2 = .16) in women with prior DEP. Neither delta ALLO at 60 minutes nor a history of abuse served as an independent predictor of symptom severity in PMDD women with prior DEP, and no variable predicted premenstrual fatigue. None of the selected predictor variables predicted premenstrual symptoms in PMDD women with no prior DEP (all P values>.15).

CHAPTER 4
DISCUSSION

The results of our study indicate that histories of DEP in women both with and without PMDD are associated with alterations in ALLO responses to stress. Women with prior DEP displayed a blunted ALLO stress response at both 30 and 60 minutes following the onset of mental stress, relative to women with no prior DEP. In addition, ALLO levels in women with prior DEP did not show the expected decrease from venipuncture stress to extended baseline rest that was evident in women with no prior DEP. Taken together, these results suggest that in women with histories of depressive episodes, even in the absence of current DEP, there is a failure of ALLO mechanisms to respond appropriately to challenge as evidenced by lack of an increase in response to mental stressors and lack of a recovery following venipuncture stress.

This study is novel in several ways, including being the first to examine the impact of lifetime depressive episodes on ALLO concentrations and reactivity to stress. Prior research has focused on patients with current depression, finding decreased ALLO concentrations in depressed patients compared to non-depressed controls (Nappi et al., 2001; Romeo et al., 1998; Strohle et al., 2000; Strohle et al., 1999; Uzunova et al., 1998), though no study has examined ALLO responses to stress in depressed patients. Evidence that reduced ALLO in patients with current depression plays a pathophysiological role comes from studies showing negative correlations between ALLO levels and the severity of depression (Nappi et al., 2001; Uzunova et al., 1998) and studies showing that clinically efficacious treatment with

selective serotonin reuptake inhibitors is associated with increases in ALLO (Strohle et al., 2000; Strohle et al., 1999; Uzonova et al., 1998). The antidepressant-like effect of ALLO is also well recognized in animal models, since associations between low ALLO levels and symptoms of anxiety and depression in animal paradigms have been well established (Frye et al., 2002; Khisti et al., 2000a; Khisti et al., 2000b; Uzunova et al., 2003; Uzunova et al., 2004). In addition, rats under chronic social isolation stress, a phenomenon that is commonly seen in human depression (Prince et al., 1997; Roberts et al., 1997), exhibit lower ALLO levels than group-housed rats (Serra et al., 2000), further reinforcing the pathophysiological link between ALLO and depression.

While we did not observe differences between women with prior DEP versus no prior DEP in absolute ALLO concentrations when ALLO was sampled under situations analogous to other studies (i.e. right after venipuncture), suggesting that absolute concentrations may normalize with the remission of depression, we did obtain evidence that a prior history of DEP may have long-term repercussions on ALLO responsivity to challenge as well as on biochemical pathways. For example, in addition to the alterations in ALLO responsiveness to mental stress and failure to recover from venipuncture stress, we also observed lack of a positive correlation between ALLO and progesterone concentrations in women with prior DEP. This finding is consistent with a recent report in women with current postpartum depression who also failed to show the expected relationship between ALLO and progesterone that was seen in euthymic postpartum women (Nappi et al., 2001). These findings may reflect a differential conversion of 3α-reduced neuroactive steroids in DEP (Strohle et al., 2000; Strohle et al., 1999). Specifically, the enzyme 5α,-reductase catalyzes the reduction of progesterone into the 5α,-pregnane steroids 5α,-dihydroprogesterone (5α, -

DHP) and 5α,-dihydrodeoxycorticosterone (5α, -DHDOC). The pregnane steroids may be further reduced to the neuroactive steroids ALLO or 3α, 5α,-tetrahydrodeoxycorticosterone (3α, 5α,-THDOC) by the enzyme 3α-hydroxysteroid oxidoreductase (Strohle et al., 2000; Strohle et al., 1999). Studies by Strohle et al. (2000; 1999) have found higher plasma concentrations of the neuroactive steroid 3α, 5α, -THDOC coupled with lower plasma concentrations of ALLO in patients with current depression compared with non-depressed controls. These results may indicate differential alterations in the biosynthesis of deoxycorticosterone or it's metabolites in depressed patients.

Thus, to the extent that alterations in the expected relationship between progesterone and ALLO reflect alterations in the biochemical pathway involved in the conversion of progesterone to neuroactive steroids, our results suggest that alterations in the conversion of the 3α-reduced neurosteroids may persist beyond remission of the depressive episode. Future studies assessing both ALLO and 3α, 5α, -THDOC in women with histories of depression but who were in full remission would be needed to clarify this issue. Whether alterations in the biochemical conversion of progesterone to ALLO contributes to the dysregulation in stress responsiveness of ALLO that we have documented in women with prior DEP or whether it represents a separate dysregulation in ALLO mechanisms also remains to be clarified.

Regardless of mechanism, reduced ALLO concentrations in patients with current depression (Nappi et al., 2001; Romeo et al., 1998; Strohle et al., 2000; Strohle et al., 1999; Uzunova et al., 1998), and blunted ALLO reactivity to stress that we documented in women with histories of DEP is consistent with HPA axis abnormalities seen in depression. It is well established that a large proportion of depressed patients are hypercortisolimic (Gillespie and Nemeroff, 2005) and show exaggerated HPA-axis responses to mental stress (Young et al.,

2004; Heim et al., 2000). In animal models, ALLO prevents CRF release and gene expression in the hypothalamus as well as CRF-induced anxiety (Patchev et al., 1994), and also significantly attenuates the elevation of plasma ACTH and corticosterone following stress (Guo et al., 1995; Patchev et al., 1996). Thus, blunted ALLO function may be an associated feature of the hypercortisolimia seen in depression, though studies examining both ALLO and HPA-axis factors in patients with depression would be needed to examine this hypothesis.

It is not clear why we failed in the present study to replicate the finding of differences in ALLO concentrations or responses to stress as a function of PMDD status in the present study that were observed in our earlier study (Girdler et al., 2001). Indeed, the literature on ALLO levels in PMDD women has been mixed. One limitation to many of these prior studies (Rapkin et al., 1997; Schmidt et al., 1993; Wang et al., 1996), including our own (Girdler et al., 2001), is the failure to assess or control for lifetime psychiatric histories, given the evidence for a link between PMDD and prior depressive disorders (Pearlstein et al., 1990). If previous studies included a greater proportion of PMDD versus non-PMDD controls with histories of depression, that could contribute to the evidence for reduced ALLO concentrations in PMDD samples (Lombardi et al., 2004, Monteleone et al., 2000; Rapkin et al., 1997) or blunted ALLO stress reactivity that we previously reported (Girdler et al., 2001). Thus, it may not be PMDD per se, but lifetime depression that contributes to the blunted ALLO function that has been documented in PMDD samples. Further evidence that a history of depression, and not PMDD per se, is the primary modulator of ALLO dysfunction is indicated by our multiple regression analyses showing that only in PMDD women with prior DEP did greater ALLO concentrations at extended baseline rest, reflecting

failure to recover from venipuncture stress, and more blunted ALLO reactivity to mental stress predict worse premenstrual symptoms. ALLO failed to predict symptoms in PMDD women with no prior DEP.

Another possibility for the lack of difference in the present study between PMDD and non-PMDD women in ALLO reactivity to stress may be related to the sampling intervals that we employed. The literature on time course to peak plasma ALLO responses to stress in animal models is sparse, but suggests that plasma ALLO peaks at 70 minutes following the onset of stress (Purdy et al., 1991). In the study by Genazzani et al. (1998), where human ALLO responses to an endocrine CRH challenge were measured in plasma every 15 minutes for 120 minutes, peak plasma ALLO was found at 60 minutes post-challenge. We hypothesize that our sampling intervals, though based on animal (Purdy et al., 1991) and human studies (Genazzani et al., 1998), may have missed the peak ALLO response to mental stress since the women with no history of DEP showed only a moderate increase in ALLO from baseline to 30 and 60 minutes post-stress, and since their increase was only 20% of what we observed in the non-PMDD controls in our prior study when ALLO was sampled at 17 minutes post-stress (Girdler et al., 2001). An alternative, though not mutually exclusive possibility, is that the blunted ALLO reactivity to stress found in women with prior DEP may reflect delayed recovery from venipuncture stress, so that concentrations sampled at 30 and 60-minute post-stress are lower than pre-stress levels.

In conclusion, and regardless of mechanism, the group with a diagnosis of lifetime depression clearly fails to show any evidence for stress-induced increases in ALLO, at least at the sampling intervals that we used. The directional difference in the stress response between women with histories of DEP relative to women with no prior DEP, whether due to

differences in time course of the stress response, alterations in biochemical pathways involved in the conversion of progesterone to ALLO, or some other mechanism, may have pathophysiological relevance. This is supported by animal studies showing that ALLO plays a role in modulating physiological and/or behavioral responses to stressors (Steimer et al., 1997; Purdy et al., 1991), and by our finding that the degree of decrease in ALLO at 30 minutes post-stress accounted for 16% of the variance in premenstrual depression scores, but only in PMDD women with histories of DEP. Thus, studies examining ALLO mechanisms in women with lifetime depression as well as in PMDD populations continue to be warranted, though investigations into the time course of the ALLO stress response in healthy individuals are needed to inform future investigations into the role of ALLO reactivity to stress in psychiatric illness.

Table 1. Mean (+SEM) Demographic and Psychiatric History Information as a Function of Prior Depression and Group

	PMDD Women		Non-PMDD Women	
	Prior DEP (n = 14)	No prior DEP (n = 12)	Prior DEP (n = 17)	No prior DEP (n = 22)
Age	33.1 (1.8)	30.3 (1.9)	35.9 (1.6)	33.9 (1.4)
BMI[A]	27.1 (1.4)	22.4 (1.5)	24.5 (1.2)	25.1 (1.1)
Progesterone level (ng/mL)	15.1 (2.5)	15.6 (2.7)	19.0 (2.3)	15.9 (2.0)
% Minorities	35.7	25	17.6	27.3
No. Prior Anxiety (%)	3 (21%)	2 (18%)	*5 (29%)	0 (0%)
No. PTSD (%)	3 (21%)	1 (9%)	0 (0%)	0 (0%)
No. Eating Disorders (%)	*5 (36%)	0 (0%)	2 (10%)	0 (0%)
No. Substance Abuse or Dependence (%)	2 (14%)	1 (9%)	*5 (29%)	1 (5%)

BMI = Body Mass Index, DEP = depression

[A] PMDD with prior DEP > PMDD with no prior DEP, $p<.05$

* Percent greater than other cells, $p < .05$

Table 2. Predictors of Premenstrual Symptoms in PMDD Women with a History of DEP (N=14) on the Basis of Multiple Regression Analyses

Predictor Variables	Depression	Irritability	Labile Mood	Fatigue
Luteal ALLO Following Extended Baseline	R^2 =.37 β=.61	R^2 =.30 β =.55	R^2 =.34 β=.58	--
Luteal Delta ALLO at 30 min Post-Stress	R^2 =.16 β= -.40	--	--	--
Luteal Delta ALLO at 60 min Post-Stress	--	--	--	--
History of Abuse	--	--	--	--
Total Model R^2	R^2=.54 F(2,11) = 5.2 $p < .05$	R^2=.30 F(1, 11) = 4.3 $p = .06$	R^2 =.34 F(1,11) = 5.2 $p < .05$	--

-- indicates that the predictor variable did not account for significant variance in premenstrual symptom

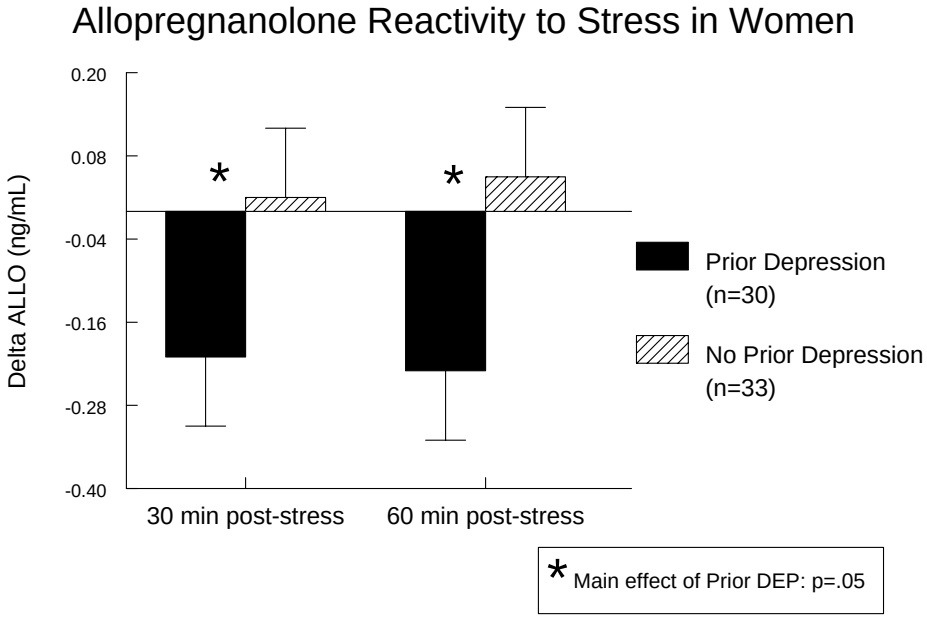

Figure 1: Change (stress – baseline) in allopregnanolone (ALLO) concentrations at 30 and 60 minutes post-stress in women as a function of histories of depression (DEP).

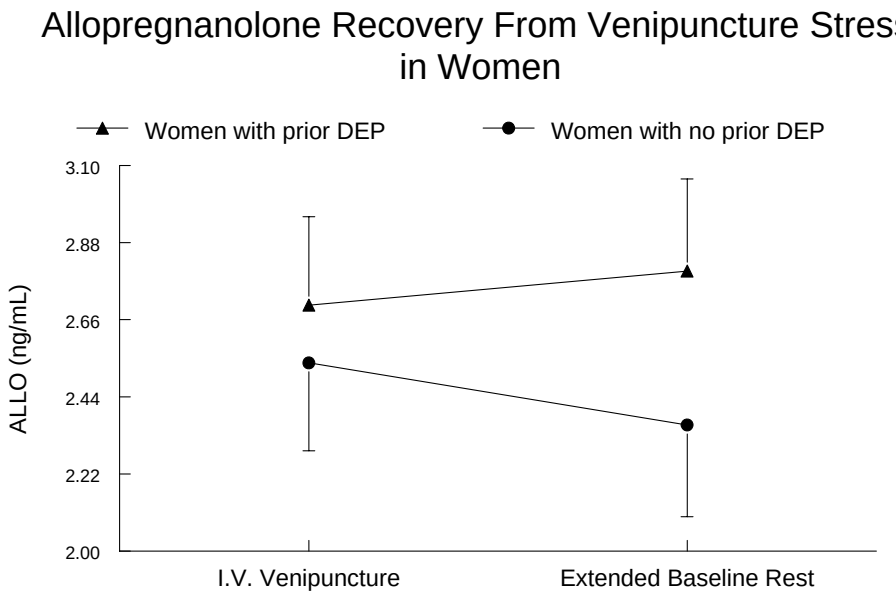

Figure 2: Allopregnanolone (ALLO) concentrations taken immediately after intravenous (i.v.) venipuncture and again after an extended baseline rest in women as a function of histories of depression (DEP).

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