

Cortical γ -Aminobutyric Acid Levels Across the Menstrual Cycle in Healthy Women and Those With Premenstrual Dysphoric Disorder

A Proton Magnetic Resonance Spectroscopy Study

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Background: There is increasing support for the hypothesis that gonadal steroids involved in the regulation of the human menstrual cycle modulate γ -aminobutyric acid (GABA) neuronal function. This study tests the hypothesis that cortical GABA neuronal function, reflected in brain GABA concentrations, fluctuates across the menstrual cycle in healthy women and those with premenstrual dysphoric disorder (PMDD) and that a menstrual cycle phase-dependent abnormality in brain GABA concentrations in women diagnosed as having PMDD would reflect altered central response to circulating gonadal and neuroactive steroids.

Methods: Fourteen healthy menstruating women and 9 women diagnosed as having PMDD were recruited from a women's behavioral health research program located at a university-based medical center. The women underwent serial proton magnetic resonance spectroscopic measurements of occipital cortex GABA levels across the menstrual cycle (primary outcome measure) and had blood drawn for gonadal hormone and neurosteroid levels determined on each scan day (secondary outcome measure).

Results: There was a significant group \times phase interaction with most of the finding explained by the reduction in cortical GABA levels during the follicular phase in those with PMDD compared with healthy controls. Cortical GABA levels declined across the menstrual cycle in healthy women, whereas women with PMDD experienced an increase in cortical GABA levels from the follicular phase to the mid luteal and late luteal phases. Significant between-group differences in the relationship between hormones and GABA were observed for estradiol, progesterone, and allopregnanolone.

Conclusions: These data strongly suggest that the GABAergic system is substantially modulated by menstrual cycle phase in healthy women and those with PMDD. Furthermore, they raise the possibility of disturbances in cortical GABA neuronal function and modulation by neuroactive steroids as potentially important contributors to the pathogenesis of PMDD.

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PRECLINICAL STUDIES have increasingly defined the neural targets for gonadal steroids, such as estradiol and progesterone, and their neurosteroid precursors and derivatives.¹⁻⁴ For example, the 3α -reduced biosynthetic derivatives of progesterone, 3α -hydroxy- 5α -pregnan-20-one (allopregnanolone) and 3α -hydroxy- 5β -pregnan-20-one (pregnenolone), are potent γ -aminobutyric acid A ($GABA_A$) receptor facilitators, increasing the frequency and duration of the chloride ionophore channel opening.⁴ In healthy women, menstrual cycle-related changes in cognitive function, mood, and drug sensitivity may be attributable to fluctuations in the hormonal modulation of γ -aminobutyric acid (GABA) systems.⁵⁻⁷

Alterations in GABA neuronal function have been implicated in the patho-

physiology of premenstrual dysphoric disorder (PMDD), a luteal phase-specific syndrome characterized by moderate-to-severe alterations in mood, behavior, and physical well-being that impairs the personal, professional, and/or social functioning of 3% to 7% of premenopausal women.⁸ Plasma GABA levels are reduced across the menstrual cycle in women with PMDD compared with healthy controls who experience an increase in plasma GABA levels from the follicular to luteal phases.⁹ Additionally, women with PMDD demonstrate a luteal phase-specific decrease in the behavioral and physiologic response to administration of $GABA_A$ receptor agonists.^{6,7} Measurement of cortical excitability using transcranial magnetic stimulation demonstrates an increase in cortical inhibition in healthy controls in the mid luteal phase compared with the

Table 1. Participant Characteristics*

Characteristic	PMDD (n = 9)	Controls (n = 14)
Age, y	34.6 ± 4.50	30.1 ± 6.23
Age at menarche, y	12.1 ± 1.62	11.9 ± 1.71
Age of PMDD onset, y	25.0 ± 4.91	NA
Cycle length, d	28.9 ± 4.81	28.64 ± 3.30
DSRP score, follicular	34.9 ± 6.5	25.1 ± 2.6
DSRP score, luteal	66.1 ± 25.4	25.3 ± 2.3

*Data are presented as mean ± SD. PMDD indicates premenstrual dysphoric disorder; DSRP, Daily Record of Severity of Problems; and NA, not applicable.

mid follicular phase to a degree comparable to that observed after administration of GABA-enhancing drugs.¹⁰ The fact that women with PMDD did not demonstrate an increase in cortical inhibition during the mid luteal phase provides additional evidence of phase-specific reduction in GABA_A receptor function¹¹ in this population. Preclinical findings demonstrating alterations in the number of $\alpha 4$ subunits and sensitivity of GABA_A receptor on withdrawal of chronically administered allopregnanolone¹² provide a compelling model for the mechanism by which neurosteroids may mediate GABA neuronal changes across the menstrual cycle.

Further support for a neurosteroid-GABA hypothesis in the pathogenesis of PMDD comes from treatment studies that demonstrate the preferential responsiveness of PMDD to selective serotonin reuptake inhibitors (SSRIs). The SSRI-induced increases in cerebrospinal fluid and brain allopregnanolone in patients with major depression¹³ and in rats,¹⁴ respectively, are likely due to the stimulatory effect of SSRIs on 3 α -hydroxysteroid oxidoreductase, the rate-limiting enzyme in the biosynthesis of allopregnanolone.¹⁵ Although luteal phase deficits in allopregnanolone are associated with greater symptom severity in women with PMDD,^{16,17} most studies have found no difference between women with premenstrual syndrome and PMDD and their healthy counterparts with respect to luteal phase levels of gonadal steroids and allopregnanolone.¹⁷⁻¹⁹ Failure to demonstrate a significant effect of diagnosis on plasma neuroactive steroids does not necessarily detract from the importance of these steroids in the pathogenesis of PMDD because a dissociation between peripheral and brain levels of allopregnanolone has been demonstrated in rodents.^{20,21}

Quantitative, localized, repeatable, noninvasive measurements of human cortical GABA levels are now possible.²² These advances in proton magnetic resonance spectroscopy (¹H-MRS) permit direct tests of the hypothesis that human cortical GABA levels fluctuate across the menstrual cycle and that PMDD is associated with abnormalities in GABA neuronal function. The aims of this study are 3-fold: (1) to determine whether there are menstrual cycle phase- and diagnosis-specific fluctuations in cortical GABA levels in normal menstruating women and women with PMDD, (2) to assess menstrual cycle-related fluctuations in plasma neuroactive steroids, and (3) to examine the relationship between plasma neuroactive steroids and cortical GABA levels. We chose the

occipital cortex as our region of interest for this study based on previous evidence that GABAergic-modulating substances (benzodiazepines, alcohol, vigabatrin) and psychiatric disorders (unipolar depression and panic disorder) were associated with altered GABA levels in this region.²³⁻²⁵

PARTICIPANTS AND METHODS

PARTICIPANTS

Twenty-three regularly menstruating women, 9 with PMDD (age, 26-39 years; mean age, 34.6 years) and 14 healthy controls (age, 21-45 years; mean age, 30.1 years), participated in this study after giving written informed consent using forms and procedures approved by the Yale University School of Medicine Human Investigations Committee, New Haven, Conn. All participants were recruited through local advertising and were paid for their participation. Participants had not been pregnant or taking hormonal contraceptives for at least 10 months and had not taken psychotropic drugs for more than 5 years. Two participants with PMDD had previously taken fluoxetine hydrochloride for major depressive episodes.

All participants underwent a diagnostic interview using the Structured Clinical Interview for Diagnosis based on DSM-IV.²⁶ Women with PMDD were excluded if they had an Axis I psychiatric or substance abuse or dependence disorder within the previous 2 years or a lifetime history of bipolar disorder or psychosis. Healthy controls were without personal or family (first-degree relative) history of a confirmed psychiatric disorder (by participant report). **Table 1** summarizes participant characteristics. Participants did not differ significantly with respect to age at presentation, age at menarche, parity, or menstrual cycle length.

All women were screened prospectively for 2 to 3 consecutive menstrual cycles using the Daily Record of Severity of Problems to rate severity of mood and physical symptoms.²⁷ The Daily Record of Severity of Problems is a 24-item daily diary that solicits information regarding the severity of mood, cognitive, behavioral, and physical symptoms and level of interference in the personal, professional, and interpersonal life domains. Each symptom is rated on a scale of 1 (not present) to 6 (extreme). All women with PMDD had at least a 50% increase in severity of at least 4 mood symptoms in the luteal phase (average score for 7 days before onset of menses) compared with the follicular phase (average score days 5-11). No symptom was rated higher than a 3 (mild) on days 5 to 11, and 4 symptoms were rated at least a 4 (moderate) in severity for at least 2 of the 7 days before onset of menses.

TIMING OF ¹H-MRS SCANS

Scans were scheduled to coincide with the period of low gonadal steroid levels (days 3-8; early to mid follicular phase), highest gonadal steroid levels (3-8 days after luteinizing hormone surge; mid luteal phase), and the period of gonadal steroid withdrawal (1-5 days before onset of menses; late luteal phase). Timing of the luteinizing hormone surge was determined using a commercially available urine luteinizing hormone kit (Answer, Carter-Wallace, New York, NY). Blood was obtained for gonadal steroid and neurosteroid determination on each scan day. Serum was assayed for estradiol and progesterone levels by a commercial laboratory (Clinical Laboratory Partners, Hartford, Conn) to confirm menstrual cycle phase. The GABA data were not included in any statistical analysis that included menstrual cycle phase (operationalized group) when hormone levels could not confirm the desired phase. Follicu-

lar phase scan data from 1 participant with PMDD and 2 healthy controls were excluded from phase-specific analyses secondary to having estradiol levels of more than 100 pg/mL (367 pmol/L) (indicative of late follicular phase). Data from 2 mid luteal phase scans (1 PMDD participant, 1 healthy control) were omitted from analyses because of a progesterone level of 299 ng/dL (9.5 nmol/L) or less.

All women participating in the study abstained from drinking alcohol for at least 48 hours before each ¹H-MRS scan and had minimal (none to 3 drinks per week) alcohol use at baseline with the exception of one participant with PMDD who regularly drank 10 glasses of wine per week. One participant with PMDD reported smoking 5 to 7 cigarettes per day but abstained from smoking for at least 5 hours before each scan. Daily ratings during the scan month continued to confirm PMDD diagnosis for those included in the study.

MRS METHODS

Studies were performed with a 2.1-T magnet (Oxford Magnetic Technology, Oxford, England) with a 1-m bore and a spectrometer (Bruker Avance; Bruker Instruments, Billerica, Mass). Participants lay supine with the occipital cortex against an 8-cm surface coil tuned to the ¹H-MRS frequency of 89.67 MHz. Gradient-echo scout images of the participant's brain were obtained for participant positioning, and a 1.5 × 3 × 3-cm³ voxel centered on the midline of the occipital cortex, 1.5 cm deep from the dura, was chosen for ¹H-MRS. Automated first- and second-order shimming was applied in the volume of interest.²⁸ Detection of the 3.0 ppm of GABA C4 resonance was performed for 20 minutes using J-editing.²² Briefly, pairs of subspectra were obtained, one with a frequency selective inversion pulse applied to the GABA C3 resonance and one without the inversion pulse. The subspectra were subtracted to obtain difference spectra that contained total GABA (combined measure of GABA and the GABA-containing dipeptide homocarnosine). Localization was achieved with selective excitation, 3-dimensional, image-selected, in vivo spectroscopy, outer volume suppression, and a surface spoiler coil. The spectral acquisition parameters were as follows: repetition time, 3.39 seconds; echo time, 68 milliseconds; sweep width, 1500 Hz; and acquisition time, 510 milliseconds. The free induction decay was zero-filled to 32 K, processed with 3-Hz lorentzian broadening, and Fourier transformed. The GABA signal was then integrated over a 0.30-ppm bandwidth at 3.00 ppm, and the creatine signal was integrated over a 0.20-ppm bandwidth at 3.00 ppm in the GABA-inverted spectrum. The GABA concentration was calculated as described previously.²² **Figure 1** depicts representative spectra obtained during the follicular phase from 1 participant with PMDD and 1 healthy control using ¹H-MRS.

GONADAL STEROID AND NEUROSTEROID DETERMINATION

Serum estradiol and progesterone levels were determined in a commercial laboratory using heterogeneous competitive magnetic separation assay with a lower limit of detection (LOD) of 30.7 pmol/L and immunoassay techniques with a within-run coefficient of variation of 8.1% at the LOD for estradiol and an LOD of 0.32 nmol/L and coefficient of variation of 9.3% for progesterone. Neurosteroids, including pregnenolone, 5 α -dihydroprogesterone (5 α DHP), and allopregnanolone, were determined using gas chromatography–mass spectroscopy (GC-MS) modified from Hubbard et al.²⁹

GC-MS Conditions

The mass spectrometer was a Perkin Elmer Turbomass (negative chemical ionization mode). The gas chromatograph (GC)

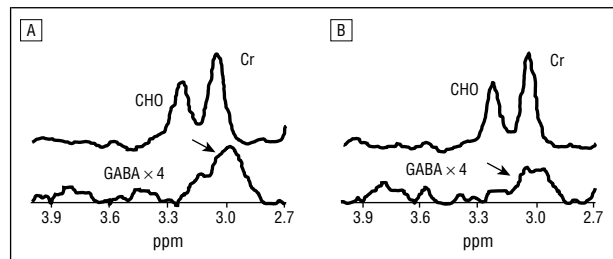


Figure 1. Example of γ -aminobutyric acid (GABA) spectra obtained from the occipital cortex during the follicular phase of 1 healthy control (A) and 1 woman with premenstrual dysphoric disorder (B). The arrows indicate the GABA peak. Cho indicates choline; Cr, creatine; and ppm, parts per million.

was an Autosystem XL (Quadrex Corp, New Haven, Conn) equipped with a methylsilicone column (15 m × 0.25 mm inside diameter) with a 0.05- μ m film thickness. The initial gas chromatograph oven temperature was 150°C, followed by 230°C at 30°C/min, then to 250°C at 1.0°C/min, and finally to 320°C at 30°C/min. Ultrahigh-purity helium was the carrier gas. The gas chromatograph was operated in the splitless mode for the first 0.7 min and then was switched to a split flow. The injection port flow was 1.0 mL/min. Methane was used as the reagent gas for negative chemical ionization. The injector and transfer-line temperatures were maintained at 300°C and 310°C, respectively. Selected ions of m/z (mass/charge) 460, m/z 462, and m/z 466 were monitored.

Steroid Extraction and Separation

Steroids in plasma were extracted by solid phase extraction using C18 columns. For plasma samples, the internal standard solution was added, followed by an aliquot of methanol and water (proportion, 50/50), then diluted with deionized water to a final concentration of 5% methanol. Samples were then extracted with C18 columns equilibrated with methanol. The steroid fraction was eluted with methanol, then evaporated to dryness at 40°C.

Steroids were derivatized according to the method of Hubbard et al.²⁹ and were reacted in the following order: 50 μ L of 2% carboxymethylamine hemihydrochloride in pyridine at 60°C for 45 minutes, a volume of 25 μ L each of 20% pentafluorobenzyl bromide in acetonitrile and 20% diisopropylethylamine in acetonitrile at 45°C for 20 minutes, and 25 μ L of acetonitrile and 50 μ L of bis(trimethylsilyl)trifluoroacetamide at 40°C for 30 minutes. After each step, the reaction mixture was dried under nitrogen.

All neurosteroids for participants with PMDD and healthy controls were batch run under the same laboratory conditions. The ranges of between-day coefficient of variation for duplicate repeats of samples performed throughout several months were 3.6% to 15% for 5 α DHP, 5.5% to 27% for pregnenolone, and 8.4% to 22% for allopregnanolone.

STATISTICAL METHODS

Mixed models were used to analyze the data on GABA and neuroactive steroid levels.^{30,31} This approach takes into account correlations between repeated measurements on the same participant and is unaffected by data missing at random. Before analysis, all outcomes were checked to ensure that they resembled a normal distribution using normal probability plots and Kolmogorov-Smirnov test statistics. Log transformations were used when data exhibited positive skewness.

Differences in GABA levels across the 3 phases between the 2 groups (aim 1) were tested in a computer program (SAS PROC MIXED; SAS Institute, Cary, NC) by fitting a mixed model

Table 2. Cortical γ -Aminobutyric Acid (GABA) and Peripheral Gonadal Hormone and Neurosteroid Levels Across the Menstrual Cycle in Women With Premenstrual Dysphoric Disorder (PMDD) and Healthy Controls*

	PMDD	Controls	P Value
GABA, mmol/kg			
Follicular	0.78 ± 0.23	1.67 ± 0.25	<.001
Mid luteal	1.27 ± 0.55	1.15 ± 0.32	.65
Late luteal	1.27 ± 0.32	1.12 ± 0.40	.41
Group × phase analysis	<.001
Progesterone, nmol/L			
Follicular	2.50 ± 1.48	2.41 ± 1.37	.92
Mid luteal	31.34 ± 11.73	44.59 ± 25.79	.37
Late luteal	23.37 ± 13.27	8.94 ± 8.38	.01
Group × phase analysis01
Estradiol, pmol/L			
Follicular	183.04 ± 95.08	180.81 ± 59.08	.89
Mid luteal	436.35 ± 230.66	463.39 ± 169.41	.63
Late luteal	365.90 ± 134.26	230.06 ± 147.91	.02
Group × phase analysis03
Pregnenolone, nmol/L			
Follicular	0.82 ± 0.71	0.83 ± 0.80	.98
Mid luteal	1.79 ± 1.08	0.88 ± 0.62	.07
Late luteal	1.69 ± 1.07	0.54 ± 0.33	.01
Group × phase analysis03
Allopregnanolone, nmol/L			
Follicular	1.02 ± 0.75	1.56 ± 0.62	.10
Mid luteal	4.75 ± 3.47	6.80 ± 2.83	.32
Late luteal	4.90 ± 1.67	4.55 ± 3.72	.81
Group × phase analysis33

*Data are presented as mean ± SD. Ellipses indicate data not applicable. To convert progesterone to ng/dL, divide by 0.0318; and estradiol to pg/mL, divide by 3.67.

with a random effect for participant and fixed effects for group, phase, and group × phase interaction. If the group × phase interaction effect was significant at the .05 level, follow-up individual comparisons between the groups at each phase were performed. Bonferroni correction was used for multiple post hoc comparisons.

Similarly, overall and phase between-group differences for each gonadal steroid and neurosteroid (aim 2) were tested by fitting a separate mixed-effects model with the same fixed and random effects described herein. Because phase was an effect in these models, the analyses were performed on the operationalized sample (the results were essentially the same for the entire sample).

The relationship between GABA and each steroid (aim 3) was estimated by fitting a separate mixed model with GABA as the response variable, with a random effect for participant and fixed effects for group, steroid, and group × steroid interactions. Because phase and steroids are usually highly correlated, phase was not used as an effect in this model, and the analysis was performed on the entire sample. When a significant difference in the relationship between GABA and a gonadal steroid or neurosteroid was observed (a significant group × steroid interaction), a graph with regression line for each group was created to illustrate the effect.

RESULTS

GABA data were obtained in the follicular phase for 8 women with PMDD and 12 healthy controls, in the mid luteal phase for 7 women with PMDD and 11 healthy controls, and in late luteal phase for 7 women with PMDD and 9 healthy controls. Reasons for not obtaining GABA measurements at all 3 time points include inability to coordinate scanner availability with participant's men-

strual cycle or schedule (n=4), participant movement in the scanner (n=1), and dropout after the second scan (n=1). Most women underwent scanning first in the follicular phase; however, 3 healthy controls underwent scans in an alternate sequence. One healthy control had follicular and late luteal phase scans in one cycle and the mid luteal phase scan in a subsequent cycle 2 months later. There was no significant between-group differences in the timing of the mid luteal (t test; $t_{11}=0.69$, $P=.51$) and late luteal phase ($t_9=1.67$, $P=.13$) scans with respect to the number of days after luteinizing hormone surge.

MENSTRUAL CYCLE FLUCTUATIONS IN GABA LEVELS

Mean GABA, gonadal steroid, and neurosteroid levels are given in **Table 2**. For the operationalized sample, there was a significant group × phase interaction ($F_{2,25}=17.9$, $P<.001$) for the cortical GABA data, explained mainly by a significant difference in follicular phase GABA levels ($F_{1,25}=27.8$, $P<.001$) (**Figure 2**). These findings continued to be significant when age was added as a covariate or when those undergoing scanning in an alternate sequence were removed from the analysis. There were no significant group differences in the mid luteal phase ($F_{1,25}=-0.20$, $P=.65$) or the late luteal phase ($F_{1,25}=0.71$, $P=.41$) (Table 2).

Examining GABA data by group, healthy menstruating women experienced a significant decrease in cortical GABA levels from the follicular phase to both the mid luteal phase ($F_{1,25}=18.2$, $P<.001$) and the late luteal phase ($F_{1,25}=21.2$, $P<.001$). In contrast, a signifi-

cant increase in cortical GABA from the follicular phase to both the mid luteal phase ($F_{1,25}=8.9, P<.006$) and the late luteal phase ($F_{1,25}=9.2, P=.005$) occurred in the women with PMDD. There was no significant between-group difference in the absolute magnitude of the change in cortical GABA levels from the follicular to mid luteal phase ($t_{11}=0.69, P=.51$).

To examine potential effects of subtle between-group differences that may have been imposed by scanning women at different points in the luteal phase with respect to their peak hormone levels and variation in menstrual cycle length, we examined the GABA data with the day of cycle scanned standardized to a 28-day cycle (day of cycle scanned/cycle length \times 28). GABA data based on the standardized day of cycle variable were analyzed using a mixed-effects model with a random intercept and a random slope for participant and fixed effects for group, day, and group by day. The findings from this analysis again demonstrated a significant group \times day interaction ($F_{1,11}=39.5, P<.001$). History of major depression ($n=3$) did not seem to have an impact on cortical GABA levels.

MENSTRUAL CYCLE FLUCTUATIONS IN GONADAL STEROIDS AND NEUROSTEROIDS

Sufficient data were obtained for estradiol, progesterone, pregnenolone, 5 α DHP, and allopregnanolone to allow for meaningful analysis of impact on cortical GABA levels and/or between-group differences in hormone levels. All variables except 5 α DHP were log transformed to eliminate positive skewness. Results for progesterone, estradiol, pregnenolone, and allopregnanolone are given in Table 2. With respect to between-group differences in hormone levels, significant group \times phase interaction occurred for pregnenolone ($F_{2,26}=3.7, P=.03$), progesterone ($F_{2,25}=5.0, P=.01$), and estradiol ($F_{2,25}=3.96, P=.03$). Between-group differences for these hormones occurred in the late luteal phase (pregnenolone: $F_{1,26}=7.9, P=.009$; progesterone: $F_{1,25}=10.3, P=.004$; and estradiol: $F_{1,25}=6.77, P=.02$), although the late luteal difference in estradiol did not pass the conservative Bonferroni correction. Analysis of between-group difference in 5 α DHP levels was limited to the mid luteal phase secondary to limited sensitivity of the assay to detect follicular phase levels. Women with PMDD had significantly higher levels of 5 α DHP in the mid luteal phase than healthy controls ($F_{1,12}=5.3, P=.04$).

RELATIONSHIP BETWEEN PLASMA NEUROACTIVE STEROIDS AND CORTICAL GABA LEVELS

Significant between-group differences in the relationship between hormones and GABA were observed for estradiol ($F_{1,31}=13.1, P=.001$), progesterone ($F_{1,31}=13.8, P=.001$), allopregnanolone ($F_{1,31}=8.7, P=.01$), and the progesterone-allopregnanolone ratio ($F_{1,31}=5.9, P=.02$). Pregnenolone and the pregnenolone-allopregnanolone ratio had no significant overall or by group impact on GABA levels. As follow-up to the significant interaction tests, separate regression lines were estimated for healthy controls

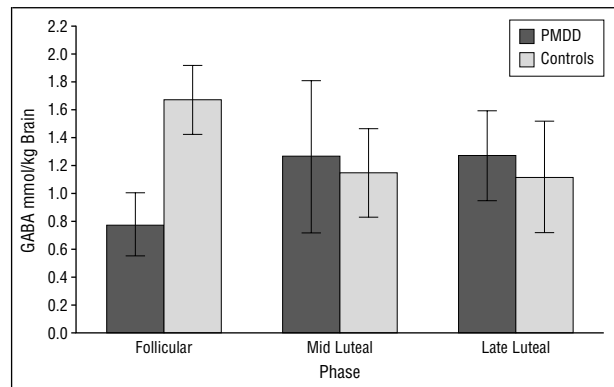


Figure 2. Proton magnetic resonance spectroscopic findings of cortical γ -aminobutyric acid (GABA) levels across the menstrual cycle in women with premenstrual dysphoric disorder (PMDD) and healthy controls. Error bars indicate SD.

and participants with PMDD and are shown in **Figure 3**. Estradiol ($\beta=-.23$; 95% confidence interval [CI], $-.42$ to $-.05$), progesterone ($\beta=-.12$; 95% CI, $-.21$ to $-.04$), and allopregnanolone ($\beta=-.19$; 95% CI, $-.34$ to $-.05$) were significantly negatively associated with GABA levels in healthy controls, whereas estradiol and progesterone were significantly positively associated with GABA levels in participants with PMDD (for estradiol: $\beta=.36$; 95% CI, $.08$ -. $.63$; and for progesterone: $\beta=.13$; 95% CI, $.02$ -. $.25$). The relationship between cortical GABA levels and allopregnanolone in the PMDD group was not significant ($\beta=.12$; 95% CI, $-.04$ to $.28$).

COMMENT

To our knowledge, this report presents the first direct measurement of the cortical level of a neurotransmitter across the menstrual cycle in healthy women and women with PMDD. The most striking finding in this study was the effect of diagnosis on the menstrual cyclicity seen in cortical GABA levels in both groups. Cortical GABA levels decreased across the menstrual cycle in healthy women, whereas the opposite occurred in women with PMDD. These findings were not altered by participant age, whether the data were analyzed for the operationalized group or for the entire sample, or whether the data were analyzed based on day of cycle instead of menstrual cycle phase. Women with PMDD have reduced cortical GABA levels during the follicular phase, but they are not significantly different from healthy counterparts during the mid or late luteal phase. These findings are consistent with those of Schmidt et al,³² who suggest that the pathophysiologic processes responsible for the symptoms associated with PMDD may not be restricted to the late luteal phase. Furthermore, the magnitude of the change in GABA levels from follicular to mid luteal phase was significant in both groups, suggesting that most of the group \times phase finding cannot be accounted for by cortical GABA changes in one participant group alone.

Factors other than diagnosis or menstrual cycle phase that may have influenced our findings include potential for instability in the GABA measurement or, alternatively, the presence of a first scan effect. Each of these alternative explanations is improbably based on previ-

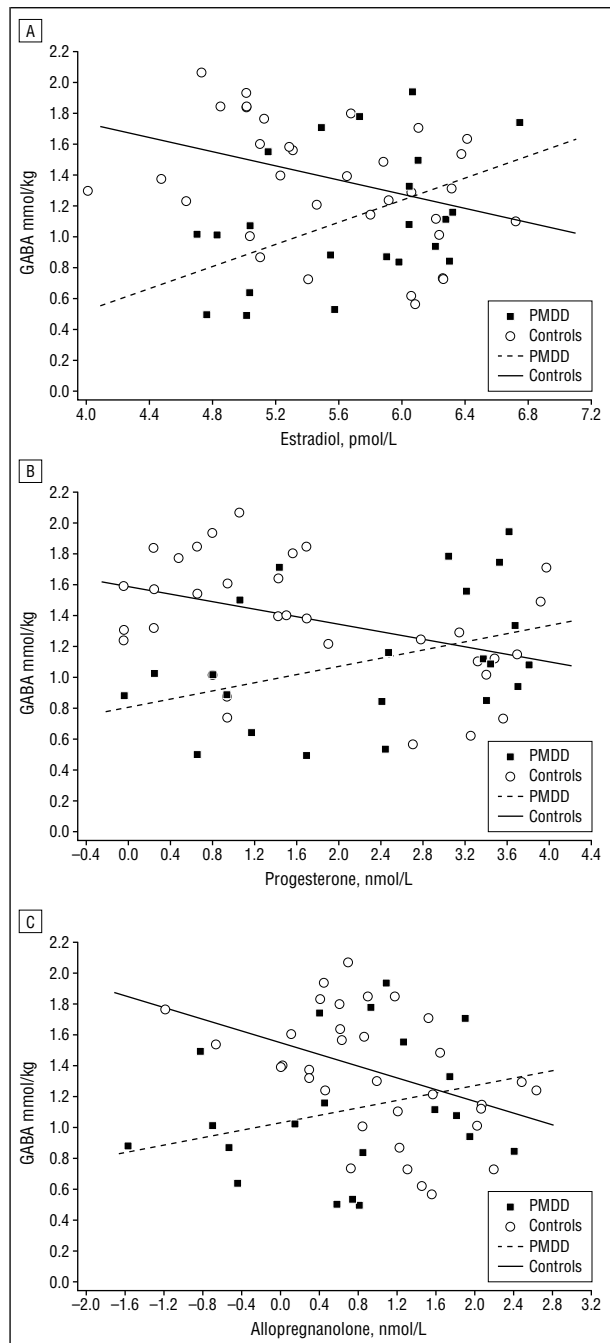


Figure 3. Mixed-model analyses of covariance revealed significant between-group differences in the relationship between the following hormones (on log scale) and γ -aminobutyric acid (GABA): estradiol (A) ($F_{1,31}=13.1$, $P=.001$), progesterone (B) ($F_{1,31}=13.8$, $P=.001$), and allopregnanolone (C) ($F_{1,31}=8.7$, $P=.01$). In the healthy controls, estradiol ($\beta=-.23$; 95% confidence interval [CI], $-.42$ to $-.05$), progesterone ($\beta=-.12$; 95% CI, $-.21$ to $-.04$), and allopregnanolone ($\beta=-.19$; 95% CI, $-.34$ to $-.05$) were negatively correlated with cortical GABA levels. In the group with premenstrual dysphoric disorder (PMDD), there was a positive correlation between cortical GABA levels and estradiol ($\beta=.36$; 95% CI, $.08$ – $.63$) and progesterone ($\beta=.13$; 95% CI, $.02$ – $.25$) and no significant relationship with allopregnanolone ($\beta=.12$; 95% CI, $-.04$ to $.28$). To convert estradiol to pg/mL, divide by 3.67; progesterone to ng/dL, divide by 0.0318.

ous data that the interscan variation in cortical GABA levels in men who underwent scanning several times in 1 month is 0.10 mmol/kg brain.²² Furthermore, the group \times phase findings for the GABA data were un-

changed when women who underwent scanning in an alternate sequence were removed from the analysis.

The decline in cortical GABA levels from the follicular to luteal phase seen in healthy controls in the present study is in contrast to the plasma GABA findings of Halbreich et al⁹ and suggests that peripheral measures of GABA function may not accurately reflect central function. Likewise, we did not detect a difference in cortical GABA levels in those women with PMDD and a history of major depression compared with those without such history; however, our sample size may have been a limiting factor.

The relationship between cortical GABA levels and symptoms in PMDD is in contrast to that of major depression and panic disorder, where symptomatic episodes are associated with reduced occipital cortex GABA.^{24,25} Our follicular phase GABA levels in the PMDD group (mean \pm SD, 0.78 ± 0.23 mmol/kg brain) were substantially lower than those reported for women with major depression (melancholic subtype) (1.10 ± 0.66 mmol/kg brain)^{33,34} and a group of men and women with panic disorder (1.08 ± 0.30 mmol/kg).²⁴ In contrast, during the mid and late luteal phases when our participants were symptomatic, their GABA levels were not unlike those of the healthy controls in the present study. Although these findings suggest that follicular phase GABA levels distinguish women with PMDD from those with major depression and support the diagnostic distinction between major depression and PMDD, these findings need to be confirmed in a study of cortical GABA levels in MDD in which menstrual cycle phase is clearly defined. Of interesting, our finding of a decrease in cortical GABA levels in healthy controls as the endogenous levels of the neurosteroid GABA agonists rise is consistent with ¹H-MRS findings demonstrating a reduction in cortical GABA levels in healthy controls after a single oral dose of 0.5 mg of the GABA agonist clonazepam.²⁴

The fact that allopregnanolone levels did not distinguish women with PMDD from healthy counterparts is consistent with most studies¹⁷⁻¹⁹ but not all.^{35,36} Our finding of higher late luteal phase estradiol and progesterone levels in the PMDD group can be contributed to having studied a greater proportion of these women (50% vs 33%) more than 3 days before the onset of menstrual flow.

In the present study, the relationship between gonadal steroids and cortical GABA levels in healthy women was opposite to that seen in those with PMDD. For healthy women, the rise in estradiol, progesterone, and allopregnanolone levels in the mid luteal phase signaled a decrease in cortical GABA levels that persisted, despite hormonal declines in the late luteal phase. This pattern would be consistent with the hypothesis that these or related hormones directly or indirectly depress GABA synthesis, perhaps via their facilitation of GABA_A function. However, cautious interpretation of this finding is warranted for several reasons. First, it is curious that estradiol, which is a GABA_A antagonist^{1,2} and would be expected to oppose the GABA neuronal effects of the 3 α -reduced progesterone metabolites, appeared in this study to have either the same directional effect or a weaker antagonistic effect on GABA levels. Second, the gonadal steroid levels, which were determined in a commercial laboratory,

are known to increase in a predictable fashion from the follicular to mid luteal phase. These expected hormonal fluctuations may have enhanced the likelihood of obtaining a significant correlation between hormones and GABA levels, giving the appearance of driving the GABA levels down or up in the healthy controls and participants with PMDD, respectively.

With these caveats considered, the rise in cortical GABA levels across the menstrual cycle and the positive correlation between gonadal steroid levels and cortical GABA levels in participants with PMDD could have several explanations. First, PMDD may be associated with an abnormality in GABA_A receptor function that conveys attenuated GABA neuronal sensitivity to the inhibition-enhancing effects of agents that typically facilitate GABA_A receptor function. This hypothesis would be consistent with the literature suggesting a luteal phase-specific decrease in cortical inhibition^{11,12} and reduced sensitivity to the behavioral and physiologic effects of a benzodiazepine⁶ and the neurosteroid pregnanolone⁷ administration in women with PMDD. Our finding that allopregnanolone had a significant impact on cortical GABA levels in healthy controls but not those with PMDD is consistent with these findings. Preclinical studies¹² suggest this phenomenon may be secondary to allopregnanolone-induced expression of the $\alpha 4$ subunit of GABA_A, which conveys reduced sensitivity to the facilitatory effects of agonists for this receptor.

This study parallels other human studies that indicate that benzodiazepine administration²⁴ and inhibition of GABA catabolism²⁵ depress GABA levels, presumably by depression of the expression or function of the GABA synthetic enzyme glutamic acid decarboxylase 67 (GAD₆₇). Continuing with this theory, these findings suggest that feedback inhibition of GAD₆₇ by GABA_A receptor agonists outweighs the stimulatory effects of estradiol on GAD₆₇ activity reported in some brain regions in rodents.^{37,38} For example, the rise in cortical GABA levels across the menstrual cycle could reflect the induction of factors that reduce GABA_A receptor function in the luteal phase and contribute to anxiety in patients with PMDD.

However, this study has several limitations that influence interpretation of its results. This study was unable to differentiate whether fluctuations in cortical GABA levels reflect changes in GABA synthesis or GABA turnover. This distinction may become possible as carbon 13 MRS techniques are developed for directly measuring these processes.³⁹ The naturalistic design of this study limits the ability to determine the impact of individual hormones on cortical GABA levels. This could be rectified by conducting ¹H-MRS studies in menstruating women using gonadotropin-releasing hormone agonists with add-back paradigm to isolate the effects of individual hormones on cortical GABA levels. Finally, although the occipital cortex receives input from many cortical and limbic regions, it is not generally implicated in the pathogenesis of mood disorders. Therefore, the findings of this study may be "downstream" from the neural circuitry of mood regulation.

In summary, this study documented menstrual cycle-related changes in cortical neurochemistry in healthy women. Future studies will be needed to further characterize the nature and functional consequences of this

phasic GABAergic modulation in healthy women. This finding also highlights the importance of incorporating menstrual cycle phase within clinical research designs involving menstruating women.

This study also begins the important and complicated process of mapping the neurochemical determinants of PMDD. Premenstrual dysphoric disorder presents unique challenges to the study of brain chemistry. Postmortem studies of cortical neurotransmitter systems in PMDD are probably impossible for several reasons: (1) valid diagnosis requires prospective assessment during multiple menstrual cycles; therefore, retrospective assessments from unintentional deaths are not likely to be useful; (2) life-threatening illnesses generally impair the regulation of the menstrual cycle and therefore prevent the use of brain tissue collected from young women; and (3) the onset of menopause prevents the use of brain tissue collected from elderly patients. Therefore, in conjunction with a solid foundation in preclinical research, molecular brain imaging, despite its limitations, seems uniquely suited for exploring the neurochemistry of PMDD.

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