Published in final edited form as:

Pain. 2013 April ; 154(4): 548–559. doi:10.1016/j.pain.2012.12.019.

The role of circulating sex hormones in menstrual cycle dependent modulation of pain-related brain activation

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Abstract

Sex differences in pain sensitivity have been consistently found but the basis for these differences is incompletely understood. The present study assessed how pain-related neural processing varies across the menstrual cycle in normally cycling, healthy females, and whether menstrual cycle effects are based on fluctuating sex hormone levels. Fifteen subjects participated in four test sessions during their menstrual, mid-follicular, ovulatory, and midluteal phases. Brain activity was measured while nonpainful and painful stimuli were applied with a pressure algometer. Serum hormone levels confirmed that scans were performed at appropriate cycle phases in 14 subjects. No significant cycle phase differences were found for pain intensity or unpleasantness ratings of stimuli applied during fMRI scans. However, lower pressure pain thresholds were found for follicular compared to other phases. Pain-specific brain activation was found in several regions traditionally associated with pain processing, including the medial thalamus, anterior and mid-insula, mid-cingulate, primary and secondary somatosensory cortices, cerebellum, and frontal regions. The inferior parietal lobule, occipital gyrus, cerebellum and several frontal regions demonstrated interaction effects between stimulus level and cycle phase, indicating differential processing of pain-related responses across menstrual cycle phases. Correlational analyses indicated that cycle-related changes in pain sensitivity measures and brain activation were only partly explained by varying sex hormone levels. These results show that pain-related cerebral activation varies significantly across the menstrual cycle, even when perceived pain intensity and unpleasantness remain constant. The involved brain regions suggest that cognitive pain or more general bodily awareness systems are most susceptible to menstrual cycle effects.

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The authors declare no conflicts of interest.

Keywords

circulating hormones; pain sensitivity; pain-related brain activation; menstrual cycle; functional brain imaging

1. Introduction

Sex differences in pain have been consistently reported in both clinical and experimental literature. In general, women are more likely to develop chronic pain syndromes and are more sensitive to experimental pain than men [2,12,20,52]. The basis for these sex differences is however still poorly understood [16]. Gonadal steroid hormones are thought to be important factors related to sex differences in pain sensitivity. This notion is supported by numerous studies demonstrating differences in pain sensitivity over the menstrual cycle [14,18,26,29,49]. A meta-analysis of the literature indicated that sensitivity is lowest at the follicular phase for most pain modalities [38] but inconsistent findings have been reported in more recent studies [23,25]. These conflicting findings may be partly explained by the large variation in standardizing cycle phases, and the frequent omission of blood serum analyses to verify menstrual cycle stage [40].

Menstrual cycle studies aim to investigate the influence of naturally fluctuating sex hormone levels on pain sensitivity measures. Few studies have directly examined the associations between circulating sex hormone levels and menstrual cycle effects on pain sensitivity measures. Although increased progesterone and estrogen levels were found to be correlated to increased pain in one report [39], no such associations were found in other studies [23,25]. Studies examining the combined effects of estrogen and progesterone also report mixed results; progesterone may counteract the pronociceptive effects of estrogen [21], but the opposite has been also found [44]. Recently, menstrual cycle dependent changes in experimental pain were found to be only minimally related to hormonal variations [48].

Circulating sex hormones may modulate pain-sensitivity by affecting neural activity. Sex differences in pain-evoked brain activity have been reported in multiple studies [3,10,11,19,22,32,34,46], but the cortical representation of pain across the menstrual cycle has received little attention. Significantly higher heat pain-related activation was found in a low versus a high estrogen condition -without specific mention of the representing menstrual cycle phase- in several brain regions, while more activation in the high estrogen condition was found in only one brain region, the right posterior cerebellum [9]. Another study reported significantly higher heat pain-related activation for the thalamus in the luteal compared to the follicular phase, and for several other regions in the follicular compared to the luteal phase [7]. Interestingly, in this study thalamic activation in the luteal phase correlated positively with pain unpleasantness ratings and negatively with testosterone levels. In general, it remains unclear whether menstrual cycle variations in nociceptive processing exist and what the underlying neural mechanisms are due to the small number of studies, methodological difficulties, and inconsistent findings.

The aim of the current study was to assess menstrual cycle effects and corresponding circulating sex hormone levels on the pain-related brain activation using functional magnetic resonance imaging (fMRI) in healthy, normally cycling, females. We included four different menstrual cycle phases representing distinct physiological conditions: menstrual, mid-follicular, ovulatory, and midluteal phases. This approach enabled us to examine whether the different menstrual cycle stages adequately represent fluctuating hormonal effects.

2. Methods

Participants

Fifteen normal cycling healthy females completed study participation. Four additional subjects started study participation but did not complete the study. Reasons for drop-out were time commitment (n=2), painful tooth problems requiring analgesic medication (n=1), and commencing birth control medication use (n=1). Subjects were recruited by flyers which were posted in public areas at the University of Maryland, Baltimore, and surrounding regions. Eligible participants were females between 18-45 years of age who were right-handed and spoke English fluently. Further, participants reported, and were determined to have, a normal recurrent menstrual cycle of 25-35 days in which ovulation and menstruation took place. This was documented by their filling out daily menstrual cycle diaries for two months prior to the start of data collection. Finally, participants were eligible when they were willing and able to undergo MRI scanning, and they adequately perceived pressure stimuli as painful and non-painful as determined in a pre-fMRI screening and training session. Exclusion criteria included: 1) cognitive impairment that prevented understanding the consent form or test instructions, which was assessed by the Mini Mental State Examination (MMSE; cut-off score that was used: 29 for those with college education [8]); 2) health problems as assessed by a health questionnaire including history of drug or alcohol abuse, psychiatric disorder or dysfunction requiring treatment, history of abnormal electrocardiogram, pulmonary disease, chronic respiratory disease, hypertension, heart or artery disease including heart failure and stroke, renal disease, seizure disorders, endocrine disorders such as thyroid and diabetes, chronic pain, arthritis, insomnia, reproductive system problems such as endometriosis, carpal tunnel syndrome, undergoing chemotherapy or radiation treatments; 3) obesity (Body Mass Index (BMI) ≥ 30); 4) if the painful stimulation failed to elicit a rating of 60 on a 0-100 visual analogue scale of pain intensity; 5) unable to undergo MRI scanning; 6) pregnancy; 7) use of psychotropic medications during the preceding 6 months; 8) use of hormone therapy including hormonal birth control pills during the preceding month; 9) use of tobacco in the last 6 months; 10) having experienced any serious injury to the body regions to be tested; or 11) if regularly exercising more than 1 hour a day, three times a week. The study was approved by the University of Maryland Institutional Review Board for the Protection of Human Subjects, and all participants provided written informed consent.

Procedures

All subjects participated in a pre-fMRI screening session followed by four MRI scan sessions during different phases of their normal menstrual cycle. In the pre-fMRI screening session, subjects underwent informed consent procedures, protocol training, and a series of sensory tests of their perception of a range of pressure stimuli. Also, they filled out several questionnaires. These first series of sensory tests were performed in the mid-follicular phase for all women. After completion of this first session, the subjects were asked to keep a diary in which they noted their daily body temperature measurements, the duration of their menstruation, and the occurrence of ovulation based on ovulation test kits provided to them. Participants filled out this diary for at least two menstrual cycles before participating in the subsequent protocol. The ovulation test kit consisted of several test strips which detected the presence of luteinizing hormone (LH) in the urine. These tests were self-administered at home. Additionally, participants were asked to measure their body temperature each morning to help track their cycle and ovulation time, which aided with scheduling during subsequent cycles. An accurate oral thermometer was provided to participants for this purpose. Body temperatures were to be measured each day immediately after awakening, before any physical activity was undertaken, and recorded on their diaries. After review of these first cycle diaries, the subject was requested to keep track of her menstrual cycle and

ovulation for the duration of the study by continuing to use the diaries and ovulation kits. Subsequently, participants underwent four experimental sessions involving sensory testing and functional brain imaging during the following cycle phases: the menstrual phase (within 2-4 days of onset of the menstrual cycle), the mid-follicular phase (within 6-8 days of onset of the menstrual cycle when estrogen and progesterone levels are low), peri-ovulatory (the day of or the day after the first positive ovulatory test; about 14 days after onset of the menstrual cycle, when estrogen levels are high and progesterone still low), and during the midluteal phase (one week after the ovulatory period; around 20 days after onset of the menstrual cycle when both estrogen and progesterone levels are high). The order of testing across cycle phase was balanced among subjects. Specifically, participants were quasi-randomly assigned to have their first experimental session in one of the four phases of the menstrual cycle, such that session number and cycle phase were balanced across subjects. A urine sample was collected at each visit to test for pregnancy, as this was an exclusionary criterion. None were excluded on this basis.

Circulating sex hormones and standardization of menstrual cycle phases

Twenty cc blood samples were drawn at each test day. Samples were always obtained before scanning started. Since research time on the scanner was limited to the afternoons (after the clinical scans finished) the timing of the blood samples drawings was generally similar, with a few exceptions if a scan had to be made in the weekend due to a positive ovulation test. From these blood samples, levels of the hormones estradiol, progesterone, free testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH) were determined by immunoassays (estradiol, progesterone, and free testosterone with RIA, LH and FSH with ELISA). These serum analyses allowed for confirmation of accurate timing of menstrual cycle phases. More specifically, rises in LH and FSH are confirmatory measures of ovulation as these trigger ovulation. Testosterone was measured to examine potential cyclic variations in this hormone which is of importance as this can be metabolized into estradiol. Reference values for these hormonal levels over different phases of the menstrual cycle have been published previously [4,46]. In order to compare menstrual cycle effects between subjects, the individual cycles were converted to a standardized cycle duration of 28 days according to criteria published by LeResche et al. [28]. Briefly, the recorded LH surge day is set to be day 14 of a standard 28-day cycle. This LH surge day was identified based on the first positive test day from the ovulation test kit as indicated on each cycle diary. A cycle day less or equal to the LH surge day was standardized to: $\text{standardized cycle day} = \text{cycle day} \times 14 / \text{LH surge day}$. A cycle day greater than the LH surge day was standardized to: $\text{standardized cycle day} = 14 + (\text{cycle day} - \text{LH surge day}) \times 14 / (\text{cycle length} - \text{LH surge day})$. We added the menstrual phase of the cycle to our design as clinical pain scores have been reported to be highest around this period (e.g., for temporomandibular disorder [28]), suggestive of the fact that rapid falls in levels of progesterone and/or estrogen may lead to higher pain sensitivity.

Sensory testing

Mechanical pressure stimuli were delivered to the left foot dorsum with a hand-held algometer which consisted of a spring-controlled device delivering calibrated pressure via a flat 10mm diameter rubber tip (Wagner Instruments, Greenwich, CT). For each participant, 2 stimulus levels were selected: a non-painful stimulus evoking a distinctly innocuous pressure sensation and a stronger stimulus evoking a pain intensity rating of moderate intensity (60 ± 10 on a 0 to 100 pain intensity scale, in which 0 represented “no pain”, and 100 represented “the most intense pain imaginable”). Accordingly, the specific mechanical pressure stimulation intensities used were determined on a subject-specific basis in the pre-fMRI session such that the perception of pain intensity was equalized across subjects rather than the stimulus intensity. Participants were instructed to rate their pain intensity and pain

unpleasantness on a scale with anchors for ‘no pain’ / ‘not at all unpleasant’, and ‘most intense pain imaginable’ / ‘extremely unpleasant’. Each participant was instructed that she could signal to halt the protocol at any time for reasons of discomfort.

Questionnaires

Subjects completed several questionnaires to assess psychosocial variables that may influence pain responses. The following questionnaires were administered: the Beck Depression Inventory to assess depressive symptoms (BDI) [1], the Spielberger's State-Trait Anxiety Inventory to assess anxiety-related symptoms (STAI) [42], the Spielberger State-Trait Anger Expression Inventory to assess anger expression (STAXI) [43], the Positive and Negative Affect Scale to assess generalized positive and negative affect (PANAS) [53], the Pennebaker Inventory for Limbic Languidness to assess bodily symptoms (PILL) [35], the Fear of Pain Questionnaire-III to assess pain-related fear (FPQ) [31], the Kohn Reactivity Scale to assess reactivity (KRS) [24], the Pittsburgh Sleep Quality Index to assess sleep quality and potential insomnia problems (PSQI) [6], the Edinburgh Handedness Inventory to assess handedness (EHI) [33], and finally the Pain Catastrophizing Scale to assess catastrophic thinking about pain (PCS) [47].

Functional imaging

In the functional brain imaging sessions, brain responses to the presentation of nonpainful and painful stimuli were determined. A block design was used with a stimulus presentation of 14 seconds and an inter-stimulus interval varying among 12, 14, and 16 seconds in a random trial by trial sequence to reduce anticipatory effects and potentially confounding auto-correlative artifacts. In each run, 3 nonpainful and 3 painful stimuli were presented in a pseudo-random order with intervening baseline periods. In total, two functional runs were acquired and data were concatenated for subsequent data processing. After each functional run, subjects were asked to rate the average pain intensity and unpleasantness of the weaker and the stronger stimuli over the run. Pain ratings were averaged over the two runs. The functional MRI scans were acquired on a 3 T Tim Trio scanner (Siemens Medical Solutions, Malvern, PA) with a 12-channel head coil with parallel imaging capability. A gradient echo single shot echo-planar-imaging (EPI) sequence provided a 3.6×3.6 mm resolution over a 23-cm field of view (FOV). T2* weighting from this sequence was accomplished with an echo time (TE) of 30 ms and flip angle 90° . The repetition time (TR) was 2000 ms, allowing the whole brain to be covered using 24 slices in an interleaved manner with a slice thickness of 6 mm and no gaps between slices. A 3D T1 MPRAGE volumetric scan was acquired for anatomical reference of the functional scans with parameters: 3.44 ms TE, 2250 ms TR, 900 ms TI, flip angle 9° , 96 slices, slice thickness of 1.5 mm and a 0.9×0.9 mm in-plane resolution over a 23 cm FOV. Other scans were acquired in the same sessions, but are not reported here.

Data analysis

Functional imaging data was analyzed using Analysis of Functional NeuroImages (AFNI; <http://afni.nimh.nih.gov>). The first four volumes were removed from the functional scan series to allow for signal equilibration. The remaining volumes were corrected for slice timing differences, and were spatially aligned to the first volume for motion correction. Registration was conducted within a session (a single cycle phase). A brain-only mask was then created of the functional data set which was used for general linear model (GLM) analysis. After coregistration to the anatomical T1-weighted MRI, spiking artifacts were reduced and the time series were temporally smoothed using a moving three-point weighted average. The functional volumes were subsequently spatially smoothed using a 5-mm full-width, half-maximum Gaussian filter. Linear, second-order, and third-order trends within the time series were removed, and voxelwise normalization was achieved by dividing the signal

intensity at each time point by the voxels mean intensity. A GLM was used to model temporal responses to painful and nonpainful stimuli. Responses were modeled with a voxelwise regression of fMRI signal time courses assuming a standard boxcar regressor convolved with the hemodynamic response function with an 8 seconds lag. Regressor coefficients from the painful and nonpainful factors in the GLM model were then spatially normalized to the stereotactic space (Talairach). Within-subject analyses were performed on whole brain activation using a 2-way (menstrual cycle phase $\times$ stimulus level) repeated measures ANOVA. The spatially normalized regression coefficients for the painful and nonpainful stimuli were used as dependent measures in the ANOVA. Results from the ANOVA were corrected for multiple comparisons by using minimum cluster size thresholds as determined by the AFNI routine AlphaSim. The minimum cluster size threshold in the group analysis for the main effect of pain was 4 voxels in original space, which corresponded to 310 mm³ at an overall $p < 0.01$, with a minimum individual voxel threshold of $p < 0.01$ in each region. The minimum cluster size threshold in the group analysis for the main effect of phase and the interaction between pain and phase was 6 voxels in original space, which corresponded to 465 mm³ at an overall $p < 0.05$, with a minimum individual voxel threshold of $p < 0.05$ in each region. Group maps of voxel clusters which were commonly activated in the stimulus condition irrespective of cycle phase, cycle phase irrespective of stimulus condition, and cycle phase $\times$ stimulus interaction were created. Functionally activated regions were identified based on anatomical locations with the underlying structural images. Individual masks were created using 3dAutomask on the time series data for each subject in each phase. A group mask was then created which included voxels common to all phases of the menstrual cycle across all subjects. From there, a group 2 (pain) $\times$ 4 (cycle) ANOVA was run on voxels defined by the group mask. The resulting regions of interest (ROI's) were identified from the main effects and interactions of the ANOVA. Post hoc analyses were performed with paired t-test analyses for amplitude measure of the peak voxel for the regions that showed significant effects for the main effect of pain, the main effect of cycle, or an interaction between pain and cycle. Questionnaire data and data on pain threshold values and subjective ratings of pain intensity and unpleasantness were analyzed for cycle effects using repeated measures ANOVAs in SPSS version 15.

To evaluate the role of circulating sex hormone levels upon menstrual cycle effects of pain-related brain activation patterns, the following analyses were performed. For the follicular and luteal cycle phases, a separate stimulus intensity contrast map was generated (painful vs. non-painful stimuli). Then, the difference between these two cycle phases were calculated based on the contrast coefficients on a voxel-wise basis. These difference scores were correlated with cycle phase differences in estrogen or progesterone levels, separately. In this way, the within subject changes in pain-related brain activation could be correlated with hormone level changes between the two cycle phases. These particular cycle phase differences account for a large proportion of within-subject variability of serum hormone levels [39]. This resulted in two voxel-by-voxel correlation analysis of the whole brain: one related to estrogen levels and one related to progesterone levels. Using a voxel-wise threshold of $p < 0.01$ and cluster size 310 mm³, significant clusters were identified from these correlation maps. For each of those clusters, the peak voxel correlation coefficient was extracted from each subject's correlation map for group analysis. A similar approach was used to examine the role of circulating sex hormone levels upon menstrual cycle effects of pain-related brain activation patterns in the ovulatory and follicular phases, excluding one extreme outlier in the ovulatory phase, disproportionately affecting the analysis. This subject had very high circulating estrogen levels but still within the normal physiological range for this cycle phase. This measure was likely at the precise peak time. In addition, the correlational analyses were repeated taking the other hormone into account as a covariate of no interest since these hormones may potentially interact with each other.

A *p*-value below 0.05 was considered significant, except for post-hoc analyses that accounted for menstrual cycle phases where the *p*-value was Bonferroni adjusted to correct for multiple comparisons to below 0.0083 (0.05/6 accounting for all possible combinations).

3. Results

Subject characteristics

Data analysis was performed on 14 female volunteers as blood serum levels indicated that one subject was not tested in the appropriate cycle phases (see below). Mean age of these 14 subjects was 28.0 ± 6.2 standard deviation (SD), ranging from 22 to 40 years of age. Subjects had at least 4 years of college education, none of them suffered from a serious medical or psychiatric illness, they were good sleepers (mean 7.0 ± 0.7 SD hours a night), did not smoke, used small amounts of caffeinated drinks (mean 1.2 ± 0.8 SD per day), used alcohol moderately, if at all (mean 1.3 ± 1.0 SD glasses a week; three subjects were non-drinkers), and exercised light to moderately (at least 2 to 3 times a week for 30 minutes of walking, bike riding, gym exercises, or more demanding exercises such as running). All subjects were right-handed as assessed on the EHI (mean 83.1 ± 25.5 SD) and had high MMSE scores (mean 29.9 ± 0.4 SD, range 29-30) indicating good cognitive capabilities. The mean BMI score was 22.1 ± 2.2 SD (range 17.2-26.3) reflecting that subjects had a normal body weight in relation to height. Systolic blood pressure (mean over sessions 106.6 ± 10.8 SD), diastolic blood pressure (mean 64.5 ± 10.5 SD), pulse (mean 79.8 ± 9.5 SD), and skin temperature (mean 31.2 ± 1.6 SD) were assessed at each cycle but did not vary significantly over the phases. Further, none of these measures correlated significantly with blood serum estradiol or progesterone changes over the cycle. Four out of 14 subjects used pain medication (nonsteroidal anti-inflammatory drugs or paracetamol) within 24 hours of testing in the menstrual phase, 1 out of 14 in the follicular phase, 1 out of 14 in the ovulatory phase and 2 out of 14 in the luteal phase.

Questionnaires

All subjects scored within normal ranges on all the psychological questionnaires. Depressive symptoms, sleep quality, occurrence of physical symptoms, state anxiety, and state anger expression were assessed over the menstrual cycle. No significant differences were found for any of these variables over the cycle.

Menstrual cycle phases

Due to the randomization of phase order, more than half of the subjects had to be tested over a period of more than one menstrual cycle. No more than 3 cycles were needed in any subject to accomplish all four MRI scans, and in most cases 2 cycles were sufficient. Ovulation was adequately detected by urine ovulation tests for all subjects in each cycle. The average duration of the non-standardized menstrual cycle was 27.6 days (± 2.7 SD, range = 23-34 days). In order to describe the timing of cycle measurements across subjects, each individual cycle was normalized to a standardized cycle phase of 28 days as described above. Standardized menstrual cycle phase testing took place on average at day 3 ± 1.1 SD for the menstrual phase, at day 8 ± 1.3 SD for the follicular phase, at day 15 ± 1.4 SD for ovulation, and at day 21 ± 2.6 SD for the luteal phase.

Blood serum levels

Blood serum analysis confirmed that scans were performed at the appropriate menstrual cycle phase in all but one subject for whom the ovulation and luteal phases were not accurately estimated on the basis of the diaries and ovulation tests. Considering the remaining 14 subjects, significant cycle effects were found for estradiol ($F = 8.36$, $p < 0.01$),

progesterone ($F = 15.50$, $p < 0.001$), FSH ($F = 20.34$, $p < 0.001$), and LH ($F = 26.82$, $p < 0.001$; Figure 1). The pattern of cycle differences corresponded to those expected for the four different phases, for all hormones. No significant cycle phase effects were found for testosterone levels. The reliability of obtaining and comparing serum levels during different cycles was verified by comparing serum levels obtained at the follicular phase at screening versus the follicular phase at fMRI testing. No significant differences were found in serum levels between these two sessions.

Pressure pain assessments

Pressure pain thresholds (PPTs) were evaluated at each session prior to fMRI data collection (Figure 2). A significant main effect of cycle was found ($F = 3.53$, $p = 0.036$). Posthoc comparisons yielded a significant difference in PPTs for the follicular phase compared to the luteal phase ($F = 11.13$, $p = 0.006$) and a trend towards significance for the follicular phase versus ovulation ($F = 8.89$, $p = 0.011$) reflecting lower thresholds, thus increased sensitivity to pain, in the follicular phase compared to ovulation or the luteal phase. Considering how PPT and hormone levels change between cycle phases provides a specific evaluation of how individual hormone level changes may be related to PPT changes across the menstrual cycle. The difference scores for PPTs (follicular vs. luteal or follicular vs. ovulatory) were not significantly correlated to the difference scores for serum concentrations of estradiol ($r = 0.22$ for follicular vs. luteal, and $r = 0.11$ for follicular vs. ovulatory) or progesterone ($r = 0.16$ follicular vs. luteal) all p -values > 0.05 , suggesting that cycle phase differences in PPTs do not seem to be based on fluctuations in hormone serum levels of estradiol and progesterone.

Ratings in response to painful stimuli

We intended to administer identical pressure forces during MRI scanning over the cycle while assessing for varying pain ratings over the cycle. However, in three subjects we had to decrease the force of stimulation due to very high pain ratings ($> \text{VAS}80$) upon painful stimulation which would have made repeated stimulation in the MRI scanner difficult for the participant. For one subject, the high intensity force was decreased in the menstrual phase, for another subject in the follicular phase and in the last subject at ovulation. Nevertheless, no significant cycle phase differences were found in the forces used to elicit high intensity painful stimuli for the group as a whole ($F = 0.85$, $p = 0.43$). The mean force across all cycles that was used to give high intensity painful stimulation was $3.31 \text{ kg} \pm 1.1 \text{ SD}$ and for low intensity non-painful stimulation $0.84 \text{ kg} \pm 0.64 \text{ SD}$. Neither pain intensity ratings ($F = 0.35$, $p = 0.8$, scores ranged between 35 and 80 over the cycles on a 0-100 VAS) nor pain unpleasantness ratings ($F = 2.54$, $p = 0.07$, scores ranged between 30 and 84 over the cycles) varied significantly across menstrual cycle phases. Also, no significant correlations were found between difference scores for pain ratings and difference scores for hormone serum levels of estradiol (comparing follicular vs. luteal or follicular vs. ovulatory) or progesterone (comparing follicular vs. luteal). Correlation coefficients for pain intensity scores ranged between -0.18 and 0.16 and for pain unpleasantness between 0.14 and 0.41 with all p -values > 0.05 .

Functional brain imaging

Stimulus level / pain-related effects—Group contrast maps were used to identify differential activation for painful versus nonpainful stimulation. As expected, several areas showed stronger activation following painful versus nonpainful pressure stimulation irrespective of cycle phase (overall $p < 0.01$, corrected for cluster size, see Figure 3).

Menstrual cycle effects—Significant main effects of cycle (overall $p < 0.05$, corrected for cluster size) irrespective of the intensity of sensory stimulation were located in the superior parietal lobule, the angular gyrus, the middle temporal gyrus, multiple frontal regions, and the cerebellar vermis (Table 1). Posthoc Bonferroni corrected pairwise comparisons showed several different activation patterns for these ROIs across the menstrual cycle (Table 2; Figure 4). The cluster in the superior parietal lobule and both clusters in the middle frontal gyrus showed greater positive activation during the follicular and luteal phases, with weaker or negative responses in the other phases. For the middle temporal gyrus and the angular gyrus, negative activation was primarily found, particularly in the menstrual phase. Relatively low activation patterns were found for the superior frontal and the mid-vermis clusters, with greatest activation in the menstrual phase and in the luteal phase, respectively.

Interaction between stimulus intensity and cycle effects—An interaction between stimulus intensity and cycle effects was identified on a group contrast map with an overall $p < 0.05$ corrected for cluster size. Significant interactions were found in multiple frontal regions, the contralateral inferior parietal lobule; bilaterally in the middle occipital gyrus, and multiple regions in the cerebellum (Table 3; Figure 5). Posthoc Bonferroni corrected pairwise comparisons yielded significant cycle effects for the painful (high pressure) stimulation in most of these regions, but no significant cycle effects related to the non-painful stimuli (Table 4). As with the ROIs showing significant phase effects, patterns varied across brain regions. The bilateral posterior lobe and the contralateral inferior parietal lobule showed stronger activation for painful stimulation in follicular and luteal phases than the other phases. Several brain regions showed strong negative activation to painful stimuli, particularly in the menstrual phase (superior frontal gyrus, medial frontal gyrus, bilateral middle occipital gyrus, and ipsilateral posterior lobe; Figure 6).

Correlations with hormonal levels—We further assessed whether the cycle-related differences in pain-related activations were associated with circulating hormonal levels by calculating the correlation between the difference scores in estrogen levels and the difference in brain activation for the pain contrast effect in these same phases. The peak r voxel was derived from a whole brain analysis (see Methods). Significant correlations (all at a level of $p < 0.05$) for estrogen in the luteal versus follicular phases were found in the ipsilateral inferior frontal gyrus (Spearman's $r = 0.79$), the ipsilateral central sulcus (Spearman's $r = 0.83$), posterior cingulate cortex (Spearman's $r = 0.60$), ipsilateral and contralateral middle occipital gyrus (Spearman's $r = 0.66$ and Spearman's $r = 0.89$, respectively), and contralateral lingual gyrus (Spearman's $r = 0.86$). This same analysis, but now controlling for progesterone levels, yielded a significant effect for a cluster in the right cerebellum ($r = 0.94$) only. Significant correlations for estrogen in the ovulatory versus follicular phases were found in the left anterior insula ($r = 0.88$), and left and right precuneus ($r = 0.81$ and $r = 0.84$, respectively). This analysis was not repeated with progesterone as a covariate, as progesterone levels changed very little between these two cycle phases. Correlational analyses for progesterone yielded significant correlations (all at a level of $p < 0.001$) in the contralateral medial frontal gyrus ($r = 0.86$), contralateral precentral cortex ($r = 0.92$), ipsilateral superior parietal lobe ($r = 0.89$), ipsilateral middle occipital gyrus ($r = 0.88$), and the contralateral superior occipital gyrus ($r = 0.92$). This same analysis, but now controlling for estrogen, yielded significant clusters in the left caudate ($r = 0.93$) and the left precuneus ($r = 0.90$). All of these significant associations pointed to higher levels of estrogen or progesterone corresponding to increased pain contrasts. None of these areas that showed significant correlations overlapped with regions identified in Table 3. However, the anterior insula cluster found for estrogen correlations in the ovulatory versus follicular analysis did overlap with the insular region identified from the pain main effect (Figure 3).

4. Discussion

The cortical representation of pressure pain in the brain over four different menstrual cycle phases was examined in order to establish whether pain-related cerebral processing differed across the menstrual cycle, and whether naturally fluctuating hormonal levels modulate pain sensitivity and its related neural processing. The main findings of this study are the following: 1) lower pressure pain thresholds were found for follicular compared to other phases but no cycle-related changes in pain ratings were found, 2) cycle changes in pain-related brain activation were found for several brain regions not traditionally associated with pain perception, and 3) cycle-related changes in pain sensitivity measures and brain activation were only partly explained by circulating sex hormone levels.

Pressure pain sensitivity and related brain activation

Robust pressure pain-related activation was found in the expected brain regions as previously identified [36,50,51]. Interestingly, these brain regions did not show significant variation in activation across the menstrual cycle. This was consistent with the finding that cycle phase effects were not found for pain intensity or unpleasantness ratings during the imaging sessions. We also did not find any significant correlations between hormone serum levels and pressure pain measures, which is consistent with recent findings [48]. However, pressure pain threshold was lower in the follicular compared to the luteal and ovulatory phases, although the latter was only a statistical trend. This finding contradicts the meta-analysis results on menstrual cycle effects showing lowest pain sensitivity for the follicular phase [38], but are in line with a more recent menstrual cycle phase studies [40,48]. These recent studies, including our own, generally point to lowest pain sensitivity in the luteal phase but to inconsistent findings regarding the phase that shows highest pain sensitivity. This suggests that menstrual cycle phase effects on pressure pain sensitivity are probably weak, and likely susceptible to other factors. Another consideration is that cyclic variations in pressure pain thresholds may also depend on stimulation site [40]. In our study, we used the foot dorsum because of technical reasons in the MRI scanner, therefore our findings may not be directly comparable to other study outcomes.

Cycle phase variations in stimulation-evoked brain activation

Interestingly, menstrual cycle effects in pressure evoked activation were found in brain regions associated with cognition or general bodily awareness (several frontal regions, SPL) and motor skills (cerebellum), but not in brain regions traditionally associated with affective processing [30]. The strongest effects were seen in the contralateral middle frontal gyrus and in the contralateral superior frontal gyrus, in which activations were strongly positive in the follicular and/or luteal phases, and either weekly positive or negative in the menstrual phase. Based on the large differences between the menstrual and follicular phases, and similar effects in the follicular and luteal phases, these cycle effects do not appear to be based on circulating hormone levels at the time of testing. This does not necessarily mean that variations in estrogens or progesterone levels are irrelevant, but that cycle effects are based on additional features of neuroendocrine effects. For example, the recent history of hormone variation may be particularly important in their neural effects, not simply the levels at a single point in time, as has been suggested by observations of menstrual cycle effects upon chronic orofacial pain in which rapid estrogen withdrawal was associated with increases in clinical facial pain reports around menses and ovulation [28], and with the occurrence of migraine around menstruation [41].

Stimulus intensity dependent cycle phase variations in brain activation

A few brain regions showed cycle-dependent differences in activation specifically to the noxious level of stimulation (Tables 3 and 4). Similar to the results of cycle phase, these

brain regions are not generally associated with pain perception, with the possible exception of the inferior parietal lobe which showed cycle-phase variations specifically for the painful stimulation level.

Differential pain-related activation of the cerebellar posterior lobe over menstrual cycle phases was observed in the present study, and has been described before [9]. Cerebellar activity has been traditionally related to planning, learning, and coordination of movement [15]. Menstrual cycle effects in cerebellar activation are suggestive of hormonal modulation of motor preparation in response to a painful stimulus. Alternatively, differential activation in the posterior lobe of the cerebellum during cycle phases may indicate specific changes regarding anticipation of pain [37].

Notably, pain-related deactivation in the menstrual phase was the most commonly observed pattern among ROIs showing a significant cycle phase effect. These identified regions only partly overlap regions that have been identified to form the so-called 'default mode network' which displays task-induced deactivations independent of the nature of a task [5,13]. The deactivated brain regions are also not traditionally associated with sensory aspects of pain but have been described to be involved in pain modulation based on motivational accounts [27]. In the presence of pain, the activation of these regions is suppressed with the amount of deactivation varying over the cycle.

The cycle phase effects across the brain regions showed a similar pattern of weak positive signal change or stronger negative signal change during menstrual phase stimulation than for follicular or luteal phase stimulation. This can be seen for 5/7 brain regions showing a main effect of cycle phase (Figure 4), and 7/8 brain regions showing a significant interaction effect (Figure 6). The responses during the ovulatory phase were less consistent across these brain regions, but more often resembled the response during the menstrual phase than the other phases. Thus, this pattern could be interpreted as the weakest activations or strongest deactivations occurring during periods of steady hormone levels (e.g., mid-follicular and mid-luteal), in contrast to periods following hormone level changes.

Hormone level correlations with pain-related brain responses

The association between within-subject fluctuations in hormone levels and brain responses to noxious stimulation was examined with correlation analysis. Several brain regions showed significant positive correlations between cycle phase differences in estrogen and BOLD signal amplitude in response to painful stimuli, including areas in frontal, parietal, and occipital lobes. Similar positive correlations with differences in progesterone level were observed. For all these brain regions, higher levels of estrogen or progesterone corresponded to increased pain contrasts. However, little overlap was found with regions identified in the main pain or interaction analyses. These findings again indicate that the cycle effects that we observed are largely independent of the hormonal levels at the time of testing. It could be of additional value in future studies to conduct frequent assays of serum levels and examine a time-series relationship to pain sensitivity measures, although this would be methodologically challenging.

Study strengths and limitations

This study has several strengths. All women had carefully recorded regular menstrual cycles, verified ovulation, and reliable fluctuating estrogen and progesterone levels in the expected ranges. Potential stress related to the venipuncture did not affect our findings as this was always done well ahead of time before the psychophysical data collection and functional imaging started. No subject complained about lingering pain, swelling, or bruising after serum collection. Since the order of menstrual cycle phases was evenly

balanced across subjects, we can rule out potential order effects or adaptation/habituation effects of repeated measurements confounding cycle effects. Despite these methodological strengths, the findings should be interpreted with the following limitations in mind. First, no cycle phase related changes in psychosocial measures, such as mood, were found as have been described before [17]. Second, this study included a very healthy subject sample; it may be quite possible that results are different when less healthy individuals are examined. Third, we cannot guarantee that we obtained peak serum levels at the specific testing times, but we showed that the data were captured at the appropriate menstrual cycle phases. Fourth, in future studies it would be advisable to monitor menstrual cycle related pain and pain medication use meticulously. Finally, serum hormone levels may not reflect brain levels because of differential transfer across the blood-brain-barrier and de novo synthesis of neurosteroids, particularly progesterone and its metabolites, within brain tissue thereby limiting the use of hormone levels as covariates in the imaging results.

Conclusions

Taken together, this study demonstrated that pain-related brain activation varies significantly across the menstrual cycle in some regions, despite lack of significant variation in the level of pain evoked. Consistent with these observations, brain regions showing significant menstrual cycle effects were not associated with pain perception, but more likely with cognitive or motor function. The predominant pattern was either weaker activation or stronger negative signal change during the menstrual phase than during the follicular or luteal phases, particularly in several frontal regions. This suggests that brain regions implicated in cognitive pain modulation or more general bodily awareness are most susceptible to menstrual cycle effects. When correlating the sex hormone data with pain-related brain activations, we only found partial overlap indicating that significant cycle effects in pain-related activation are generally independent from hormonal levels.

Acknowledgments

This work was supported by P50-AR49555 (JDG), and the University of Maryland General Clinical Research Center (GCRC) Grant M01-RR16500.

References

1. Beck AT, Ward CH, Mendelson M, Mock JE, Erbaugh JK. An inventory for measuring depression. *Arch Gen Psychiatry*. 1961; 4:561–571. [PubMed: 13688369]
2. Berkley KJ. Sex differences in pain. *Behav Brain Sci*. 1997; 20:371–380. [PubMed: 10097000]
3. Berman SM, Naliboff BD, Suyenobu B, Labus JS, Stains J, Bueller JA, Ruby K, Mayer EA. Sex differences in regional brain response to aversive pelvic visceral stimuli. *Am J Physiol Regul Integr Comp Physiol*. 2006; 291:R268–276. [PubMed: 16614061]
4. Braunstein GD, Reitz RE, Buch A, Schnell D, Caulfield MP. Testosterone reference ranges in normally cycling healthy premenopausal women. *J Sex Med*. 2011; 8:2924–2934. [PubMed: 21771278]
5. Buckner RL, Andrews-Hanna JR, Schacter DL. The brain's default network: anatomy, function, and relevance to disease. *Ann N Y Acad Sci*. 2008; 1124:1–38. [PubMed: 18400922]
6. Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh sleep quality index: a new instrument for psychiatric practice and research. *Psychiatry Res*. 1989; 28:193–213. [PubMed: 2748771]
7. Choi JC, Park SK, Kim Y-H, Shin Y-W, Kwon JS, Kim JS, Kim J-W, Kim SY, Lee SG, Lee MS. Different brain activation patterns to pain and pain-related unpleasantness during the menstrual cycle. *Anesthesiol*. 2006; 105:120–127.
8. Crum RM, Anthony JC, Bassett SS, Folstein MF. Population-based norms for the Mini-Mental State Examination by age and educational level. *JAMA*. 1993; 269:2386–2391. [PubMed: 8479064]

9. De Leeuw R, Albuquerque RJC, Andersen AH, Carlson CR. Influence of estrogen on brain activation during stimulation with painful heat. *J Oral Maxillofac Surg.* 2006; 64:158–166. [PubMed: 16413884]
10. Derbyshire SWG, Nichols TE, Firestone L, Townsend DW, Jones AKP. Gender differences in patterns of cerebral activation during equal experience of painful laser stimulation. *J Pain.* 2002; 3:401–411. [PubMed: 14622744]
11. Derbyshire SWG. Gender, pain, and the brain. *Pain Clinical Updates.* 2008; XVI(3)
12. Fillingim RB, Ness TJ. Sex-related hormonal influences on pain and analgesic responses. *Neurosci Biobehav Rev.* 2000; 24:485–501. [PubMed: 10817845]
13. Fox MD, Raichle ME. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat Rev Neurosci.* 2007; 8:700–711. [PubMed: 17704812]
14. Gazarani P, Andersen OK, Arendt-Nielsen L. A Human experimental capsaicin model for trigeminal sensitization. Gender-specific differences. *Pain.* 2005; 118:155–163. [PubMed: 16202522]
15. Glickstein M, Doron K. Cerebellum: connections and functions. *Cerebellum.* 2008; 7:589–594. [PubMed: 19002543]
16. Greenspan JD, Craft RM, LeResche L, Arendt-Nielsen L, Berkley KJ, Fillingim RB, Gold MS, Holdcroft A, Lautenbacher S, Mayer EA, Mogil JS, Murphy AZ, Traub RJ, Consensus Working Group of the Sex, Gender, and Pain SIG of the IASP. Studying sex and gender differences in pain and analgesia: a consensus report. *Pain.* 2007; 132:S26–45. [PubMed: 17964077]
17. Gonda X, Telek T, Juhász G, Lazary J, Vargha A, Bagdy G. Patterns of mood changes throughout the reproductive cycle in healthy women without premenstrual dysphoric disorders. *Prog Neuropsychopharmacol Biol Psychiatry.* 2008; 32:1782–1788. [PubMed: 18721843]
18. Hellström B, Lundberg U. Pain perception to the cold pressor test during the menstrual cycle in relation to estrogen levels and a comparison with men. *Integr Physiol Behav Sci.* 2000; 35:132–141. [PubMed: 11021338]
19. Henderson LA, Gandevia SC, Macefield VG. Gender differences in brain activity evoked by muscle and cutaneous pain: A retrospective study of single-trial fMRI data. *Neuroimage.* 2008; 39:1867–1876. [PubMed: 18069004]
20. Holdcroft, A.; Berkley, KJ. Sex and gender differences in pain and its relief.. In: Wall, PD.; Melzack, R., editors. *Textbook of pain.* 5th edition. Churchill Livingstone; New York: 2005.
21. Ji Y, Tang B, Traub RJ. Modulatory effects of estrogen and progesterone on colorectal hyperalgesia in the rat. *Pain.* 2005; 117:433–442. [PubMed: 16154701]
22. Kern MK, Jaradeh S, Arndorfer RC, Jesmanowicz A, Hyde J, Shaker R. Gender differences in cortical representation of rectal distension in healthy humans. *Am J Physiol Gastrointest Liver Physiol.* 2001; 281:G1512–1523. [PubMed: 11705757]
23. Klatzkin RR, Mechlin B, Girdler SS. Menstrual cycle phase does not influence gender differences in experimental pain sensitivity. *Eur J Pain.* 2010; 14:77–82. [PubMed: 19217329]
24. Kohn, PM. Sensation-seeking, augmenting-reducing, and strength of the nervous system.. In: Spence, JT.; Izard, CE., editors. *Motivation, emotion, and personality.* Elsevier; Amsterdam: 1985.
25. Kowalczyk WJ, Evans SM, Bisaga AM, Sullivan MA, Comer SD. Sex differences and hormonal influences on response to cold pressor pain in humans. *J Pain.* 2006; 7:151–160. [PubMed: 16516820]
26. Kowalczyk WJ, Sullivan MA, Evans SM, Bisaga AM, Vosburg SK, Comer SD. Sex differences and hormonal influences on response to mechanical pressure pain in humans. *J Pain.* 2010; 11:330–342. [PubMed: 19853526]
27. Lawrence JM, Hoeft F, Sheau KE, Mackey SC. Strategy-dependent dissociation of the neural correlates involved in pain modulation. *Anesthesiol.* 2011; 115:844–851.
28. LeResche L, Mancl L, Sherman JJ, Gandara B, Dworkin SF. Changes in temporomandibular pain and other symptoms across the menstrual cycle. *Pain.* 2003; 106:253–261. [PubMed: 14659508]
29. Lugo M, Isturiz G, Lara C, Garcia N, Eblen-Zajjur. Sensory lateralization in pain subjective perception for noxious heat stimulus. *Somatosens Mot Res.* 2002; 19:207–212. [PubMed: 12396577]

30. McEwen BS, Alves SE. Estrogen actions in the cerebral nervous system. *Endocrine Rev.* 1999; 20:279–307. [PubMed: 10368772]
31. McNeil DW, Rainwater AJ III. Development of the Fear of Pain Questionnaire-III. *J Behav Med.* 1998; 21:389–410. [PubMed: 9789168]
32. Moulton EA, Keaser ML, Gullapalli RP, Maitra R, Greenspan JD. Sex differences in the cerebral BOLD signal response to painful heat stimuli. *Am J Physiol Regul Integr Comp Physiol.* 2006; 291:R257–267. [PubMed: 16601264]
33. Oldfield RC. The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia.* 1971; 9:97–113. [PubMed: 5146491]
34. Paulson PE, Minoshima S, Morrow TJ, Casey KL. Gender differences in pain perception and patterns of cerebral activation during noxious heat stimulation in humans. *Pain.* 1998; 76:223–229. [PubMed: 9696477]
35. Pennebaker, JW. *The psychology of physical symptoms.* Springer; New York: 1982.
36. Peyron R, Laurent B, Garcia-Larrea L. Functional imaging of brain responses to pain. A review and meta-analysis (2000). *Neurophysiol Clin.* 2000; 30:263–288. [PubMed: 11126640]
37. Ploghaus A, Tracey I, Gati JS, Clare S, Menon RS, Matthews PM, Rawlins JN. Dissociating pain from its anticipation in the human brain. *Science.* 1999; 284:1979–1981. [PubMed: 10373114]
38. Riley JL, Robinson ME, Wise EA, Price D. A meta-analytic review of pain perception across the menstrual cycle. *Pain.* 1999; 81:225–235. [PubMed: 10431710]
39. Ring C, Veldhuijzen van Zanten JJCS, Kavussanu M. Effects of sex, phase of menstrual cycle and gonadal hormone on pain in healthy humans. *Biol Psychol.* 2009; 81:189–191. [PubMed: 19393713]
40. Sherman JJ, LeResche L. Does experimental pain response vary across the menstrual cycle? A methodological review. *Am J Physiol Regul Integr Comp Physiol.* 2006; 291:R245–256. [PubMed: 16484434]
41. Silberstein SD. The role of sex hormones in headache. *Neurology.* 1992; 42:37–42. [PubMed: 1557190]
42. Spielberger, CD.; Gorsuch, RL.; Lushene, R. *The state-trait anxiety inventory manual.* Consulting Psychology Press; Palo Alto: 1970.
43. Spielberger, CD.; Jacobs, GA.; Russell, SF.; Crane, RS. Assessment of anger: the State-Trait Anger Scale.. In: Butcher, JN.; Spielberger, CD., editors. *Advances in personality assessment.* Vol. 2. LEA; Hillsdale: 1983.
44. Stening K, Eriksson O, Wahren L, Berg G, Hammar M, Blomqvist A. Pain sensations to the cold pressor test in normally menstruating women: comparison with men and relation to the menstrual phase and serum sex steroid levels. *Am J Physiol Regul Integr Comp Physiol.* 2007; 293:R1711–1716. [PubMed: 17652363]
45. Straube T, Schmidt S, Weiss T, Mentzel H-J, Miltner WHR. Sex differences in brain activation to anticipated and experienced pain in the medial prefrontal cortex. *Hum Brain Map.* 2009; 30:698–698.
46. Stricker R, Eberhart R, Chevailler M-C, Quinn FA, Bischof P, Stricker R. Establishment of detailed reference values for luteinizing hormone, follicle stimulating hormone, estradiol, and progesterone during different phases of the menstrual cycle on the Abbott ARCHITECT analyzer. *Clin Chem Lab Med.* 2006; 44:883–887. [PubMed: 16776638]
47. Sullivan MJL, Bishop SR, Pivik J. The Pain Catastrophizing Scale: Development and validation. *Psychol Assess.* 1995; 7:524–533.
48. Teepker M, Peters M, Vedder H, Schepelmann K, Lautenbacher S. Menstrual variation in experimental pain: Correlation with gonadal hormones. *Neuropsychobiol.* 2010; 61:131–140.
49. Tousignant-Laflamme Y, Marchand S. Excitatory and inhibitory pain mechanisms during the menstrual cycle in healthy women. *Pain.* 2009; 146:47–55. [PubMed: 19592167]
50. Tracey I, Mantyh PW. The cerebral signature for pain perception and its modulation. *Neuron.* 2007; 55:377–388. [PubMed: 17678852]
51. Treede RD, Kenshalo DR, Gracely RH, Jones AKP. The cortical representation of pain. *Pain.* 1999; 79:105–111. [PubMed: 10068155]

52. Unruh AM. Gender variations in clinical pain experience. *Pain*. 1996; 65:123–167. [PubMed: 8826503]
53. Watson D, Clark LA, Tellegen A. Development and validation of brief measures of positive and negative affect: the PANAS Scales. *J Pers Soc Psychol*. 1988; 47:1063–1070. [PubMed: 3397865]

Summary

Pain-related brain activation varies across the menstrual cycle in normally cycling, healthy females but these effects are generally independent from fluctuating sex hormone levels.

Figure 1. Mean (SD) blood serum concentrations across the menstrual phase. pg/ml, picogram per milliliter; ng/ml, nanogram per milliliter; IU, international units.

Figure 2. Pressure pain thresholds over the menstrual cycle phases. Significant at * $p < 0.0125$ or ** $p < 0.0083$.

Figure 3.

Contrast maps between painful and non-painful stimuli, irrespective of cycle phase. Brain regions with significantly different activation for painful versus nonpainful pressure stimulation are shown. The left side of each image corresponds to the right side of the brain, which is contralateral to stimulation. Functional activation is overlaid on a normalized anatomical MRI in Talairach space. The coordinates of slice cuts through the axial and coronal planes are presented in the images. In the group contrast analysis, the minimum cluster size was 4 voxels in original space, corresponding to 310 mm³ in Talairach space, at an overall $p < 0.01$.

Figure 4. Posthoc pairwise comparisons for menstrual cycle phase effects. The significant findings are irrespective of the stimulus intensity that was applied but for completeness, the amplitudes for stimulus intensity are nevertheless shown separately.

Figure 5.

Group maps for the interaction between pain-related activation and cycle-related cycle effects. Brain regions showing significant interaction effects for pain and cycle are depicted. Overlay and orientation are as described in Figure 2. In the group contrast analysis, the minimum cluster size was 6 voxels in original space, corresponding to 465 mm³ in Talairach space, at an overall $p < 0.05$.

Figure 6.
Posthoc pairwise comparisons for significant interactions between menstrual cycle phase effects and pain-related activation.

Brain regions showing main effects for cycle phase, irrespective of pain. The minimum cluster size was 465 mm³.

Table 1

Region	Side	Talairach coordinates (peak voxel)			Volume (mm ³)	Z-score
		x	y	z		
Middle frontal gyrus I	contralateral	36	21	55	528	4.29
Middle frontal gyrus II	contralateral	45	27	44	836	3.55
Superior frontal gyrus	contralateral	13	-11	54	529	3.61
Angular gyrus	contralateral	44	-59	25	683	3.51
Superior parietal lobule	contralateral	34	-65	45	484	3.51
Middle temporal gyrus	ipsilateral	-51	-63	19	883	3.33
Vermis (cerebellum)	midline	-4	-53	-10	723	3.28

Table 2

Posthoc pairwise comparisons for the significant main effects of cycle phase.

Region	Contrast	Side	t-statistic	p-value
Middle frontal gyrus I	men-fol	contralateral	-2.928	0.007
	men-lut	contralateral	-4.101	0.0003
	ovu-lut	contralateral	-4.385	0.0001
Middle frontal gyrus II	men-fol	contralateral	-2.959	0.006
	men-lut	contralateral	-3.765	0.001
Superior frontal gyrus	men-fol	contralateral	4.199	0.0003
	men-lut	contralateral	4.882	0.0001
Angular gyrus	men-lut	contralateral	-2.994	0.006
Superior parietal lobule	men-fol	contralateral	-3.068	0.005
	men-lut	contralateral	-2.847	0.008
	fol-ovu	contralateral	3.357	0.002
Middle temporal gyrus	men-fol	ipsilateral	-3.147	0.004
	fol-lut	ipsilateral	2.94	0.007
Vermis (cerebellum)	ovu-lut	midline	-3.505	0.002

Men, menstrual ; fol, follicular ; ovu, ovulation ; lut, luteal.

Brain regions showing a significant interaction effect between the main effects of pressure pain and cycle phase. Minimum cluster size was 465 mm³.

Table 3

Region	Side	Talairach coordinates (peak voxel)			Volume (mm ³)	Z-score
		x	y	z		
Medial frontal gyrus	midline	4	53	21	874	3.41
Superior frontal gyrus	ipsilateral	-15	50	42	588	3.66
Middle frontal gyrus	ipsilateral	-40	26	24	734	3.97
Inferior parietal lobule	contralateral	47	-37	39	818	3.81
Middle occipital gyrus	ipsilateral	-29	-86	15	515	3.29
	contralateral	37	-83	8	963	3.52
Posterior lobe (cerebellum)	ipsilateral	-16	-54	-44	503	3.65
	contralateral	40	-51	-37	632	3.16
Vermis (cerebellum)	contralateral	6	-50	-36	817	3.88

Table 4
Posthoc pairwise comparisons for the significant interaction effects of pressure pain and cycle phase.

Region	Contrast	Side	Stimulus level	t-statistic	p-value
Medial frontal gyrus	men-fol	contralateral	high	-3.248	0.006
Superior frontal gyrus	men-ovu	ipsilateral	high	-3.474	0.004
	men-lut	ipsilateral	high	-3.121	0.008
	fol-ovu	ipsilateral	high	-3.291	0.006
Inferior parietal lobule	men-fol	contralateral	high	-3.188	0.007
	fol-ovu	contralateral	high	4.024	0.001
Middle occipital gyrus	men-ovu	ipsilateral	high	-4.175	0.001
	men-lut	ipsilateral	high	-4.147	0.001
	men-lut	contralateral	high	-4.171	0.001
	men-lut	ipsilateral	high	-3.143	0.008
Posterior lobe (cerebellum)	ovu-lut	contralateral	high	-3.218	0.007
	men-lut	contralateral	high	3.406	0.005
Vermis (cerebellum)	fol-lut	contralateral	high	3.902	0.002