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Different Brain Activation Patterns to Pain and Pain-related Unpleasantness during the Menstrual Cycle

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Background: **The changes in the functional magnetic resonance imaging signal during anticipation, pain stimulation, and the poststimulation periods were investigated to determine whether changes in sex hormones affect brain activity.**

Methods: **Eighteen participants were examined twice, once in the follicular phase and once in the luteal phase. Half the participants were tested first during the follicular phase, and the other half were tested first in the luteal phase.**

Results: **The pain and unpleasantness ratings were significantly higher in the luteal phase than in the follicular. During the anticipation of pain, the prefrontal cortices were activated during the follicular phase, whereas the parahippocampal gyrus and amygdala were activated during the luteal phase. During the pain stimulation, putamen and cerebellum and precentral gyrus involving motor preparation and defense mechanism related to antinociceptive behavior were activated during the follicular phase, whereas the thalamus was activated during the luteal phase. During the poststimulation periods, the prefrontal cortices were activated during the follicular phase, whereas parahippocampal gyrus was activated during the luteal phase. The temporal pole was activated during the anticipation, pain stimulation, and poststimulation periods of the luteal phase.**

Conclusions: **During surgical and medical procedures, requirements of anesthetic and analgesic and anxiolytic drugs may be reduced during the follicular phase and increased during the luteal phase. These results highlight the need to consider the effects of the sex hormones in women when designing clinical or neuroimaging studies or when treating patients for pain and pain-related unpleasantness.**

PAIN is a multidimensional subjective experience that involves sensory–discriminative, cognitive–evaluative, affective–motivational, and motor–integratory components.

In women, sex hormones change cyclically. The estradiol level gradually increases during the postmenstrual follicular phase, with the highest level being reached immediately before the luteinizing hormone surge (ovulation, 14 days). Immediately after ovulation, there is an abrupt decline in the estradiol level. The estradiol level gradually increases during the early luteal phase (approximately 15 to 19 days), and the maximum rates of postovulatory estradiol secretion are attained during the advanced luteal phase (approximately 20 to 25 days). Thereafter, in the premenstrual phase (approximately 26 to 28 days), the level of estradiol secretion decreases precipitously. The progesterone levels remain low during the follicular phase of the menstrual cycle. Shortly after ovulation, the level of progesterone secretion increases steadily, peaks during the midluteal phase, and declines precipitously thereafter.[1,2] These changes in the hormone levels seem to be responsible for the cyclic modulations of certain cognitive abilities[3] and moods.[4] Many studies have examined the changes in the perceptual responses to noxious stimuli across the menstrual cycle in humans. Behavioral studies assessing the cyclic hormonal fluctuations suggest that the pain threshold is lower for most pain stimulation procedures during the luteal phase compared with the follicular phase.[2]

In the current study, we designed an anticipatory (prestimulation) period of 15-s and 30-s finger immersion stimulus at 48°C and a 30-s poststimulation period. And, we used the verbal cue ("15 s later, please immerse your middle finger into a hot bath of water [48°C]") to induce anticipatory stress (fig. 1). Expectation of potentially noxious events can increase anticipatory stress. Anticipatory stress is similar to stress before surgery and medical procedures in clinical practice. The individual responses to the same pain stimuli may differ according to individual psychological state. Psychological conditions may influence and change biologic mechanisms of pain.[5] Individuals with high levels of self-efficacy beliefs may adaptively cope with anticipatory stress and pain using a variety of coping strategies (*e.g.*, cognitive control) and may attenuate the impact of the pain stimulation. Individuals with low levels of self-efficacy beliefs may increase anticipatory anxiety and pain experience in the face of anticipatory stress and pain.[6,7] Because of changes in the sex hormones and their related central nervous system alterations during the menstrual cycle in women, individual responses to anticipatory stress and pain stimuli can differ according to the timing of pain delivery, pain intensity, interoception or exteroception of the stimulus, and controllability of pain. Until now, the relation between the menstrual cycle and pain and

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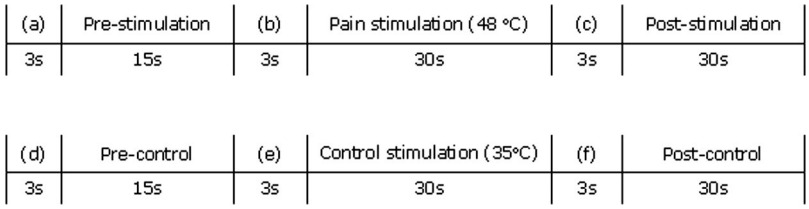

Fig. 1. Stimulation paradigm. The following instructions were given to the participants. (*A*) Fifteen seconds later, please immerse your middle finger into a hot bath of water (48°C). (*B*) Immediately, please immerse your middle finger into a hot bath of water (48°C). (*C*) Please remove your middle finger. (*D*) Fifteen seconds later, please immerse your middle finger into a tepid bath of water (35°C). (*E*) Immediately, please immerse your middle finger into a tepid bath of water (35°C). (*F*) Please remove your middle finger. This sequence was repeated five times. One repetition time (3 s) in switching between each period was excluded from the analysis to eliminate the effects of the movement of the middle finger.

pain-related unpleasantness has not been mentioned in many clinical and neuroimaging studies. To our knowledge, this study is the first to report how changes in the sex hormones between the follicular and luteal phases can influence the brain activation patterns during the pain experience.

In this study, we tested the hypothesis that cyclical changes of sex hormones and their related central nervous system alterations will change the brain activation patterns to pain and pain-related unpleasantness during the follicular and luteal phases. The blood oxygen level–dependent functional magnetic resonance imaging (fMRI) signal changes during the anticipatory stress, thermal pain stimulation, and poststimulation periods were investigated to determine whether naturally occurring changes in the sex hormones between the follicular and luteal phases affect pain intensity and brain activation during anticipation, thermal pain stimulation, and poststimulation periods.

Materials and Methods

Eighteen normal nonpregnant menstruant volunteers (aged 23.11 ±1.91 yr) were recruited from local university students and were paid for their participation. The Medical Ethics Committee of Yonsei University Wonju College of Medicine (Wonju, Kwangwon-Do, South Korea) approved this study. All of the volunteers gave written, informed consent, which acknowledged that (1) they would experience experimental thermal pain, (2) no tissue damage would result from this stimulation, (3) all of the methods and procedures were clearly explained, and (4) they were free to withdraw from the experiment at any time. All the participants were right-handed, and those with amenorrhea, irregular or recently missed menstrual periods, a previous history of any neurologic or psychiatric disorders, hormonal medication within the past 6 months, use of central nervous system–active medication or illicit drugs, or regular consumption of nicotine or alcohol were excluded. The participants were asked to refrain from smoking and alcohol and caffeine consumption for 24 h before the study. The participants were examined twice, once in the follicular phase and once in the luteal phase. Half of the participants were tested first during the follicular phase, and the other half were tested first during the luteal phase. They were also asked about their last menstrual period and the average cycle duration. The monitoring of the luteal phase was conformed by records of the next menstrual bleeding and progesterone level (progesterone: 0.15–1.4 ng/ml [follicular], 1.6–21.0 ng/ml [luteal], 5.2–23.0 ng/ml [midluteal]). The follicular or the luteal day of each participant was determined by the last menstrual period and the next menstrual bleeding as well as by the serum estradiol and progesterone level (table 1). The distal phalanges and distal two thirds of the middle phalanges of the middle finger of the left hand were immersed into an 850-ml water bath made of expandable polystyrene (at 48° or 35°C). A research assistant helped to exchange the 48°C water box for the 35°C water box.

Figure 1 shows the stimulation paradigm. The participants were informed 15 s in advance that a 48°C (pain) or 35°C (control) stimulation was to begin (anticipation period [prestimulation, precontrol, respectively]). A 30 s-finger immersion stimulation at 48° or 35°C (pain stimulation, control stimulation) was followed by a 30 s-poststimulation, postcontrol period, respectively. This 168-s block was repeated five times for each participant. The participants wore insert earphones connected to a microphone through which they received instructions during each period. Switching among each period was verbally cued. To reduce the effect of circadian fluctuations on the levels of other hormones (*e.g.*, corticosteroids), the first and second experimental session were conducted at the same time of day and conducted in an identical manner with a 2-week (nine participants) or 10-day (nine participants) interval between the two scan-

Table 1. Number of Participants for Each Follicular and Luteal Day

Follicular day	2	5	7	9	10	11	12	13
Number of participants	3	1	2	3	4	2	1	2
Luteal day	16	18	19	20	22	23	25	26
Number of participants	3	2	2	1	3	1	3	3

Follicular days and luteal days were calculated from the beginning of menstruation (day 0).

ning sessions. To minimize the level of anxiety and to familiarize the participants with the finger immersion in a hot bath, the participants underwent a 30 s-finger immersion stimulation at 48°C of the right middle finger before beginning the session. During scanning of the first three blocks of a total of five blocks, the temperature of water was decreased from 48°C to 47.5°C. We designed an interscan resting interval of 5 min between the third and fourth block. During this 5-min interval, the research assistant increased the temperature of the water from 47.5° to 48°C. On the visual analog scale, 0 indicated no sensation or no unpleasantness, and 100 indicated the most intense pain or unpleasant feeling imaginable. At an interscan interval of 5 min between the third and fourth block and the end of the stimuli series, participants were asked to rate pain intensity and unpleasantness. The pain and unpleasantness ratings were averaged for each participant. Then, a group average was acquired.

Blood Samples

A venous blood sample was drawn before each fMRI investigation to determine the estradiol, progesterone, and testosterone levels in the right forearm. The estradiol (E_2), progesterone (P_4), and testosterone levels were measured using a γ-counter (COBRA 5010 II; Packard, Downers Grove, IL) with kit Coat-A-COUNT Estradiol (DPC, Los Angeles, CA), Coat-A-COUNT Progesterone (DPC), and Coat-A-COUNT Testosterone (DPC). The intraassay and interassay coefficients of variation were 4.0 and 4.2% for estradiol, and 3.50 and 4.30% for progesterone, respectively. The coefficients of variation of testosterone were 5.90%.

Statistical Analysis of Hormone Levels and Behavioral Data

The hormonal levels of estradiol, progesterone, and testosterone and the pain and unpleasantness ratings during the follicular and luteal phases were compared using a paired *t* test. An overall *P* value of less than 0.05 was considered significant.

Functional Imaging

Before the scan, the participants were instructed to keep their eyes closed, to stay awake, and to refrain as much as possible from moving throughout the imaging session. After being placed in a comfortable position, the head was immobilized with padded ear muffs and a foam headrest, and a plastic bar was placed across the bridge of the nose. The images were acquired using a KASIT 3-T MRI scanner (ISOL Technology, Gwangju, Kyonggi-Do, Korea) with a quadrature head coil. After a T1-weighted scout image, high-resolution anatomic images were acquired using a magnetization-prepared rapid gradient echo pulse sequence with time to echo (TE) =16 ms, repetition time (TR) = 2,800 ms, flip angle = 60°, and matrix size = 192 × 256 mm. The T2*-weighted functional data were acquired using an echo planar imaging pulse sequence of TE = 30 ms, TR = 3,000 ms, flip angle = 80°, and matrix size = 64 × 64 mm. Twenty-eight slices of the echo planar images were obtained with a slice thickness of 5 mm. An experimental run consisted of a total of five blocks, and a series of tasks were performed in each block: pain (anticipation of pain stimulation − pain stimulation [48°C] − poststimulation) − control (anticipation of control stimulation − control stimulation [35°C] − poststimulation). One hundred seventy-two volumes for the first three blocks and 116 volumes for the remaining two blocks were acquired in each experimental run. The initial five images for each scan were discarded, and all images scanned during cueing were also discarded.

Imaging Analysis

The image data were analyzed using SPM99 (Wellcome Department of Cognitive Neurology, London, United Kingdom). The images for each subject were motion-corrected and realigned using the first scan as a reference. The T1 anatomical images were coregistered with the mean of the functional scans (echo planar images), which were then normalized to the SPM T1 template in the atlas space of Talairach and Tournoux. Finally, the images were smoothed by applying a gaussian filter with an 8-mm full width at half-maximum. Difference images in each participant were generated by subtracting the blood oxygen level–dependent signal data obtained during the precontrol (15 s), control stimulation (35°C, 30 s), and postcontrol (30 s) from the blood oxygen level–dependent signal data obtained during prestimulation (15 s), pain stimulation (48°C, 30 s), and poststimulation (30 s), respectively. We took the prestimulation *versus* precontrol, pain *versus* control, and poststimulation *versus* postcontrol t contrasts as the index of anticipation of pain, pain response, and poststimulation response in the follicular and luteal phase. The resulting contrast images were then used in random effect analyses at the group level. The group data for 18 participants in the follicular and luteal phase were analyzed. The brain areas activated in the follicular compared with the luteal group (n = 18, respectively) or in the luteal compared with the follicular group (n = 18, respectively) were compared using voxel-by-voxel paired *t* test. The brain activations in paired *t* test were reported if the *P* value was less than 0.005 or 0.001 (the follicular group in the poststimulation period) or 0.007 (the luteal group in the poststimulation period) uncorrected for multiple comparisons, and if the cluster size was more than 10 voxels. To acquire the simple regression between brain activation patterns and sex hormone levels (or visual analog scale scores of pain intensity and unpleasantness), the individual pain-control contrast for all participants was extracted, and sex hormone levels or visual analog scale

Table 2. Serum Levels of Sex Hormones and Behavioral Data for 18 Volunteers* in the Follicular and Luteal Phases

	Follicular	Luteal	*P* Value
Estradiol, pg/ml	55.22 ± 38.20	96.22 ± 43.88	0.013
Progesterone, ng/ml	0.87 ± 0.35	9.32 ± 5.94	< 0.001
Testosterone, ng/dl	41.28 ± 16.38	34.56 ± 15.84	0.149
Pain ratings†	37.50 ± 13.75	55.06 ± 14.70	< 0.001
Unpleasantness ratings†	32.00 ± 15.22	48.83 ± 20.23	< 0.001

Data are mean ± SD.

* Aged 23.11 ± 1.91 y. † Ratings for the visual analog scale: 0 indicated no sensation or no unpleasantness, and 100 indicated the most intense pain or unpleasant feeling imaginable.

scores of pain intensity and unpleasantness were entered into the regression model as a covariate. The resulting images were displayed on SPM99 graphic window at $P < 0.001$, with more than 10 voxels. The volume of interest with a 1-mm radius sphere at peak coordinate was selected, and the parameter estimates were acquired. The Pearson's correlation coefficients between these parameter estimates and sex hormone levels (or visual analog scale scores of pain intensity and unpleasantness) were calculated with correlation analysis using SPSS 12.0E (SPSS Inc., Chicago, IL).

Results

Hormone Levels and Behavioral Data

The estradiol and progesterone levels were significantly higher in the luteal phase than in the follicular phase (table 2). No significant differences between the follicular and luteal phases were observed for the testosterone levels (table 2). The pain ratings and pain-related unpleasantness ratings were significantly higher in the luteal phase than in the follicular phase (table 2).

Imaging Data

In the anticipation period, the activated brain regions during the follicular phase included the inferior frontal gyrus, middle frontal gyrus, medial frontal gyrus, and frontal lobe (table 3 and fig. 2A), whereas during the luteal phase, they included the cerebellum, precentral gyrus, uncus, superior temporal gyrus, amygdala, middle temporal gyrus, and parahippocampal gyrus (table 3 and fig. 2B). In the thermal pain stimulation period, the areas of brain activation during the follicular phase included the precentral gyrus, postcentral gyrus, cerebellum, occipital lobe, superior frontal gyrus, putamen, and parahippocampal gyrus (table 4 and fig. 2C), whereas during the luteal phase, they included the thalamus (right medial dorsal nucleus) and superior temporal gyrus (table 4 and fig. 2D). In the poststimulation period, the activated brain regions during the follicular phase were the superior frontal gyrus, postcentral gyrus, precentral gyrus, cerebellum, inferior frontal gyrus, and middle frontal gyrus (table 5 and fig. 2E). The brain regions activated during the poststimulation period of the luteal phase included the superior temporal gyrus, medial frontal gyrus, cerebellum, inferior frontal gyrus, precuneus, and parahippocampal gyrus (table 5 and fig. 2F).

The activation of the left precentral gyrus was negatively correlated (Pearson correlation coefficient [r] = −0.626, $P < 0.001$) with pain-related unpleasantness ratings in the pain stimulation period. The activation of the left precentral gyrus was positively correlated ($r = 0.761$, $P < 0.001$) with serum testosterone levels in the pain stimulation period of the follicular phase. There was a positive correlation ($r = 0.540$, $P = 0.001$) between activation of the right thalamus and pain-related unpleasantness ratings in the pain stimulation period. There was a negative correlation ($r = -0.524$, $P = 0.001$) between activation of the right thalamus and serum testosterone levels in the pain stimulation period.

Table 3. Brain Areas Activated in the Follicular Compared with the Luteal Group (n = 18, Respectively) and in the Luteal Compared with the Follicular Group (n = 18, Respectively) in the Prestimulation (Anticipation) Period in the Paired *t* Test

		Follicular Phase		Luteal Phase	
Region of Activation	Talairach Coordinates, x, y, z	Number of Voxels	SPM (*t*)	Number of Voxels	SPM (*t*)
Inferior frontal gyrus (R)	46, 43, 9	62	4.9		
Middle frontal gyrus (R)	40, 38, 18		3.1		
Medial frontal gyrus (R)	8, 40, 33	150	4.8		
Frontal lobe (L)	−34, 32, 21	104	4.23		
Cerebellum (R)	40, −53, −21			36	4.67
Precentral gyrus (L)	−57, −4, 43			22	4.22
Uncus (L)	−30, 3, −22			77	3.59
Superior temporal gyrus (L)	−40, 7, −19				3.24
Uncus, amygdala (L)	−24, −5, −22				3.11
Middle temporal gyrus (R)	38, 6, −32			12	3.48
Parahippocampal gyrus (R)	20, −12, −16			28	3.42

The brain activations were reported if the *P* value was less than 0.005 uncorrected for multiple comparisons, and if the cluster size was more than 10 voxels. SPM(*t*) is maximal *t* scores of significantly activated voxels. Total number of voxels of inferior frontal gyrus (right [R]) plus middle frontal gyrus (R) is 62. Total number of voxels of uncus (left [L]) plus superior temporal gyrus (L) plus uncus, amygdala (L) is 77.

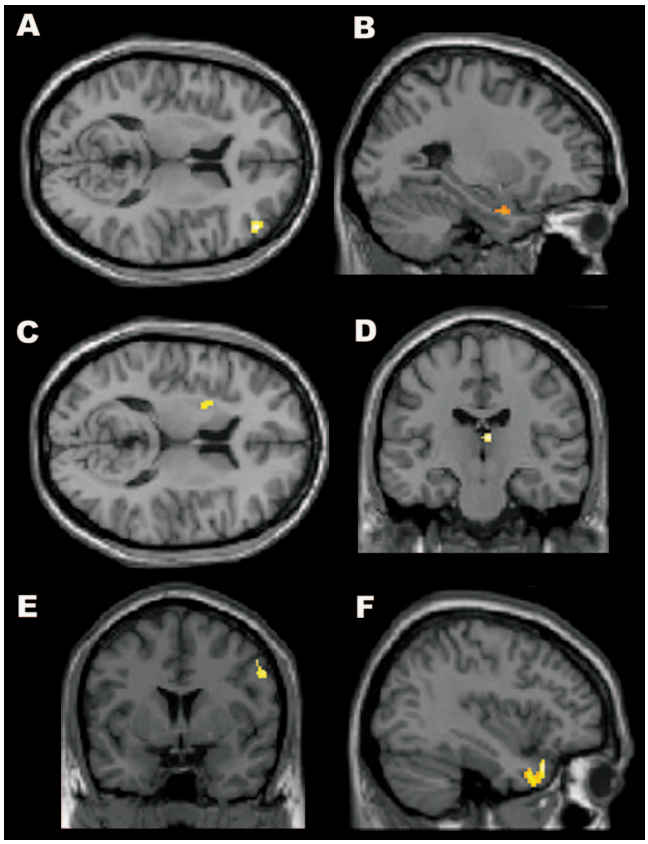

Fig. 2. Brain areas activated in the follicular compared with the luteal group (n = 18, respectively) or in the luteal compared with the follicular group (n = 18, respectively) were compared using voxel-by-voxel paired *t* test. The brain activations were reported if the *P* value was less than 0.005 or 0.001 (the follicular group in the poststimulation period) or 0.007 (the luteal group in the poststimulation period) uncorrected for multiple comparisons, and if the cluster size was more than 10 voxels. (*A*) The right inferior frontal gyrus and middle frontal gyrus in the prestimulation (anticipation) period of the follicular group. (*B*) The left uncus and amygdala in the prestimulation (anticipation) period of the luteal group. (*C*) The left putamen in the pain stimulation period of the follicular group. (*D*) The right thalamus (medial dorsal nucleus) in the pain stimulation period of the luteal group. (*E*) The right inferior frontal gyrus and middle frontal gyrus in the poststimulation period of the follicular group. (*F*) The left superior temporal gyrus (temporal pole) in the poststimulation period of the luteal group.

Discussion

In anticipation of pain, dorsolateral prefrontal cortices and right medial frontal gyrus were activated during the follicular phase, whereas the left uncus (medially curved anterior end of the parahippocampal gyrus), left superior temporal gyrus, left amygdala, right middle temporal gyrus, and right parahippocampal gyrus (part of the hippocampal complex just inferior to the hippocampus proper) were activated during the luteal phase. It may be possible that activation of the dorsolateral prefrontal cortices during the anticipation period of the follicular phase decreased pain perception during the pain stimulation period of the follicular phase. This is supported by a report that placebo analgesia which engages opioid or nonopioid mechanisms increases the brain activity of the prefrontal cortex during anticipation of pain.[8] During the anticipatory period of the follicular phase, women are likely able to earlier anticipate and adapt or cope with impending noxious events than during the luteal phase. Activation of the medial frontal gyrus and dorsolateral prefrontal cortices during the anticipation period of the follicular phase may decrease pain-related unpleasantness during the pain stimulation period of the follicular phase. There are reports that the positive emotion (presentation of happy facial expressions) activated the medial frontal cortex,[9] whereas unpleasant emotion activated the bilateral occipitotemporal cortex and cerebellum and the left parahippocampal gyrus, hippocampus, and amygdala.[10] In our study, the superior temporal gyrus (temporal pole) was activated during the anticipation period of the luteal phase. Previous studies found that increasing intensity of sad and angry facial expression was associated with enhanced activity in the temporal pole and superior temporal gyrus.[11,12] During the anticipation period of the luteal phase, the right middle temporal gyrus was activated. Recent study has shown that negative (relative to positive) autobiographical memories differentially increased neural activity in the right middle temporal gyrus only.[13] This study and our study suggest that the temporal pole and the right middle temporal gyrus may be implicated in the processing of pain-related unpleasantness. In the current study, activation of the left uncus and right parahippocampal gyrus and left amygdala during the anticipation period of the luteal phase suggests that participants during the luteal phase may feel more pain-related unpleasantness than during the anticipation period of the follicular phase. The amygdala is believed to be one of the most important brain regions for processing emotions involving fear.[14] Lorazepam (anxiolytic drug) administration attenuated the blood oxygen level–dependent fMRI signal in the bilateral amygdala in a dose-dependent manner during an emotion face assessment task.[15] This result suggests that the amygdala is activated by anxiety. Interactions between the amygdala and the hippocampal complex during emotional events have been reported.[16] Recent research showed that viewing fearful or disgusting slides increased the level of pain perception and unpleasantness, indicating that both the sensory–discriminative and affective–motivational dimensions of pain are amplified by an unpleasant emotion.[17] The anticipatory period of pain during the luteal phase could activate the brain areas that may increase pain-related unpleasantness. Inadvertent adaptation to noxious stimuli can cause anticipatory anxiety or exacerbate the emotional distress of a painful event.[18] In our experiment, women in the luteal phase were likely to have lower adaption to painful stimuli than in the follicular phase, although cerebellum and precentral gyrus involv-

Table 4. Brain Areas Activated in the Follicular Compared with the Luteal Group (n =18, Respectively) and in the Luteal Compared with the Follicular Group (n = 18, Respectively) in the Pain Stimulation Period in the Paired *t* Test

		Follicular Phase		Luteal Phase	
Region of Activation	Talairach Coordinates, x, y, z	Number of Voxels	SPM (*t*)	Number of Voxels	SPM (*t*)
Frontal-temporal space (L)	−51, 13, −2	36	4.46		
Precentral gyrus (L)	−57, 0, 7		3.05		
Postcentral gyrus (S2, L)	−53, −17, 17	309	4.4		
Postcentral gyrus (S1, L)	−46, −16, 30		4.12		
Precentral gyrus (L)	−55, −19, 36		3.93		
Cerebellum (R)	12, −65, −27	96	3.92		
Cerebellum (L)	−22, −63, 24	56	3.89		
Occipital lobe (L)	−26, −58, −2	55	3.86		
Superior frontal gyrus (L)	−24, 45, 20	30	3.62		
Cerebellum (L)	−14, −48, −25	11	3.53		
Superior frontal gyrus (R)	4, 5, 59	12	3.44		
Frontal lobe (L)	−34, 16, 14	22	3.4		
Putamen (L)	−24, 4, 11	15	3.34		
Parahippocampal gyrus (L)	−30, −24, −14	15	3.19		
Frontal lobe (L)	−26, 2, 39	12	3.14		
Thalamus (R), medial dorsal nucleus	6, −17, 12			16	3.66
Superior temporal gyrus (R)	34, 8, −29			11	3.63

The brain activations were reported if the *P* value was less than 0.005 uncorrected for multiple comparisons, and if the cluster size was more than 10 voxels. SPM(*t*) is maximal *t* scores of significantly activated voxels. S1 and S2 are the primary and secondary somatosensory cortices. Total number of voxels of frontal-temporal space (left [L]) plus precentral gyrus (L) is 36. Total number of voxels of postcentral gyrus (S2, L) plus postcentral gyrus (S1, L) plus precentral gyrus (L) is 309. R = right.

ing motor preparation and defense mechanism were activated.

During the pain stimulation period of the follicular phase, the primary and secondary somatosensory cortex (S1 and S2) were activated. It is known that both primary somatosensory cortex (S1) and secondary somatosensory cortex (S2) are commonly activated in heat pain studies and that the nociceptive input into these regions at least partially underlies perception of the sensory features of pain.[19] During pain stimulation of the follicular phase, activation of prefrontal cortices may decrease pain perception compared with the luteal phase. The putamen and cerebellum and precentral gyrus involving motor preparation and defense mechanism related to antinociceptive behavior[20] were activated during the pain stimulation of the follicular phase. The activation of these areas may facilitate the participant's effective coping with the pain stimulus. In our study, pain-related unpleasantness ratings were significantly lower in the follicular phase than in the luteal phase. The activation of the left precentral gyrus was negatively correlated with pain-related unpleasantness ratings ($r = -0.626$,

Table 5. Brain Areas Activated in the Follicular Compared with the Luteal Group (n = 18, Respectively) and in the Luteal Compared with the Follicular Group (n = 18, Respectively) in the Poststimulation Period in the Paired *t* Test

		Follicular Phase		Luteal Phase	
Region of Activation	Talairach Coordinates, x, y, z	Number of Voxels	SPM (*t*)	Number of Voxels	SPM (*t*)
Superior frontal gyrus (R)	4, 18, 51	53	5.02		
Superior frontal gyrus (R)	14, −4, 67	10	4.66		
Postcentral gyrus (S1, R)	63, −14, 30	38	4.48		
Precentral gyrus (R)	59, −13, 33				
Cerebellum (R)	12, −59, −19	16	4.27		
Inferior frontal gyrus (R)	57, 6, 33	28	3.99		
Middle frontal gyrus (R)	55, 4, 40		3.8		
Superior temporal gyrus (L)	−36, 20, −25			127	5.0
Medial frontal gyrus (L)	−6, 27, 43			18	3.57
Cerebellum (R)	4, −50, 1			23	3.49
Inferior frontal gyrus (L)	−26, 29, −3			13	3.3
Precuneus (R)	4, −54, 30			29	3.12
Parahippocampal gyrus (L)	−24, −20, 14			12	2.94

The brain activations were reported if the *P* value was less than 0.001 (follicular) or less than 0.007 (luteal) uncorrected for multiple comparisons, and if the cluster size was more than 10 voxels. SPM(*t*) is maximal *t* scores of significantly activated voxels. S1 is primary somatosensory cortex. Total number of voxels of the postcentral gyrus (S1, right [R]) plus precentral gyrus (R) is 38. Total number of voxels of the inferior frontal gyrus (R) plus middle frontal gyrus (R) is 28. In the luteal phase, because the superior temporal gyrus (left [L]) was only activated at $P < 0.001$, the threshold is decreased to $P < 0.007$. In the follicular phase, when the threshold is decreased to $P < 0.05$ with more than 10 voxels, activation of left parahippocampal gyrus and temporal pole (−36, 20, −25) is not evident.

$P < 0.001$) in the pain stimulation period and positively correlated with serum testosterone levels ($r = 0.761$, $P < 0.001$) in the pain stimulation period of the follicular phase. The activation of the left precentral gyrus increased when unpleasantness ratings decreased and serum testosterone levels increased. These results are supported by a report that endogenous testosterone or administration of testosterone increases analgesia, anxiolysis, and cognitive performance of male rats.[21] These mechanisms may explain the reason for the degree of pain and unpleasantness ratings experienced during the follicular phase being less than that in the luteal phase. However, during the pain stimulation period of the follicular phase, the activation of parahippocampal gyrus may be associated with the pain-related unpleasantness, even though the rating of unpleasantness was less than that in the luteal phase.

The right thalamus and superior temporal gyrus (temporal pole) were activated during the pain stimulation period of the luteal phase. In addition to the pain stimuli, attentional processes and vigilance have also been shown to increase thalamic activity.[22] In our study, because the thalamus was activated contralaterally to the noxious stimuli, these responses may merely reflect a sensory component that would be supposed to predominate contralaterally to the pain stimuli. However, the activation of the right thalamus was positively correlated with pain-related unpleasantness ratings ($r = 0.540$, $P = 0.001$) and negatively correlated with serum testosterone levels ($r = -0.524$, $P = 0.001$) in the pain stimulation period. The activation of the right thalamus increased when unpleasantness ratings increased and serum testosterone levels decreased. Therefore, these thalamic responses to the pain stimuli can be considered as a part of both discriminative and affective networks involved in pain processing.

During the poststimulation period of the follicular phase, the postcentral and precentral gyrus and prefrontal cortices were activated. Because the primary somatosensory cortex (S1, postcentral gyrus) was activated, residual pain could continue until the poststimulation period, although the degree of pain intensity (pain rating) was less than that experienced in the pain stimulation period. The activation of the precentral gyrus and prefrontal cortices may suggest that the motor preparation and defense mechanism related to pain modulation could continue until the poststimulation period.

During the poststimulation period of the luteal phase, the parahippocampal gyrus and temporal pole were activated. The activation of these areas may be related to an increase of pain-related unpleasantness. Also, the activation of the medial frontal gyrus in the poststimulation period of the luteal phase may be related to an increase of pain-related unpleasantness. In addition to positive emotions, negative emotions have been known to engage the medial prefrontal cortex.[23] The activation of the precuneus in the poststimulation period of the luteal phase may be associated with an increase in pain intensity and pain-related unpleasantness. Previous study reported less activation in the precuneus with hypnosis during painful heat stimuli.[24] These may suggest that the pain and pain-related unpleasantness could continue until the poststimulation period, although the degree of pain intensity and pain-related unpleasantness were less than that experienced in the pain stimulation period.

The temporal pole was activated during the anticipation, pain stimulation, and poststimulation periods of the luteal phase. The activation of the temporal pole during the entire luteal phase may be closely associated with an increase of the pain and pain-related unpleasantness. The above-mentioned results suggest that changes in the sex hormones and their related central nervous system alterations in women may decrease the feelings of pain and pain-related unpleasantness during the follicular phase and increase the feelings of pain and pain-related unpleasantness during the luteal phase.

The limitations and merits of this study are as follows. Because of the irregular menstrual cycle of some volunteers and schedules at the fMRI institute, each follicular day and luteal day for all participants could not be controlled exactly. Therefore, the same follicular day and luteal day of the 18 participants could not be compared. However, instead of comparing one point of follicular and luteal days, we could compare the follicular phase with the luteal phase throughout the menstrual cycle for 18 participants by examining each participant twice. Instead of 75-s hand immersion stimulus at 46.5°–47.5°C[25] and 30 s-hand immersion stimulus at 50°–52°C[26] used in previous reports,[25,26] 30 s-finger immersion stimulus at 48°C (a much smaller area than the hand and temperature lower than 50°–52°C, resulting in a weaker pain intensity than previous studies[25,26]) was selected because it was thought to be an appropriate thermal stimulus for detecting different responses to pain and pain-related unpleasantness between the follicular and the luteal phase. Therefore, pain-specific areas of the brain were not fully activated. However, if the temperature was increased more than 48°C and hand immersion was used, pain-specific brain regions could be fully activated, whereas we could not detect different responses to pain and pain-related unpleasantness between the follicular and the luteal phase. Because previous study has shown that brain activations continue for 20 s or so after thermal pain is removed,[8] we selected the 30-s poststimulation period. In clinical practice, this situation resembles postprocedural pain continuing after medical procedures.

In conclusion, the brain areas that may decrease pain perception were activated during the follicular phase, whereas the brain areas that may increase pain perception were activated during the luteal phase. The results of this study suggest that pain and unpleasantness may

be decreased during the follicular phase and increased during the luteal phase. During the follicular phase, women may have a higher ability to anticipate and adapt or cope with pain stimuli than during the luteal phase. Therefore, during surgical and medical procedures, requirements for anesthetic and analgesic or anxiolytic drugs may be reduced during the follicular phase and increased during the luteal phase. Although the precise, functional relation between the sex hormones and fMRI signal changes during the follicular and luteal phases requires further clarification, this study emphasizes the importance of considering the effects of sex hormones in nonpregnant menstruant women when designing clinical or neuroimaging studies or when treating patients for pain and pain-related unpleasantness.

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