Pain sensations to the cold pressor test in normally menstruating women: comparison with men and relation to menstrual phase and serum sex steroid levels

Kent Stening1,2, Olle Eriksson3, LisKarin Wahren4, Göran Berg5, Mats Hammar5, and Anders Blomqvist1

1Division of Cell Biology, Department of Biomedicine and Surgery, Faculty of Health Sciences, Linköping University, SE-581 85 Linköping, Sweden; 2Department of Health & Behavioural Sciences, University of Kalmar, SE-391 82 Kalmar, Sweden; 3Division of Statistics, Department of Mathematics, Faculty of Arts and Science, Linköping University, SE-581 83 Linköping, Sweden; 4Department of Social & Welfare Studies, Faculty of Health Sciences, Linköping University, SE-601 74 Norrköping, Sweden; 5Division of Obstetrics and Gynecology, Department of Molecular and Clinical Medicine, Faculty of Health Sciences, Linköping University, SE-581 85 Linköping, Sweden

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Correspondence to: Dr. Anders Blomqvist, Div. of Cell Biology, Faculty of Health Sciences, Linköping University, S-581 85 Linköping, Sweden. Fax: +46 13 223192. E-mail: andbl@ibk.liu.se

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ABSTRACT
The role of gonadal hormones on pain sensations was investigated in normally menstruating women (n = 16) using the cold pressor test. Tolerance time, pain threshold, and pain intensity were examined once a week during a four-week period, and serum concentrations of 17β-estradiol and progesterone were determined at each test session, which were classified into the early follicular phase, late follicular phase, early luteal phase and late luteal phase, as determined by the first day of menses and the actual hormone levels recorded. A group of men (n = 10) of the same age interval was examined for comparison. The data show that pain threshold was reduced during the late luteal phase compared to the late follicular phase, and hormone analyses showed significant positive correlation between the progesterone concentration and lowered pain threshold and increasing pain intensity. Hormone analysis also showed an interaction between s-estradiol and s-progesterone on pain intensity, demonstrating that the increased perceived pain intensity that was associated with high progesterone concentrations was significantly reduced with increasing levels of estradiol.

While no statistically significant sex differences in pain measurements were found, women displayed much more pronounced, and statistically significant, session-to-session effects than men, with increased pain threshold and decreased pain intensity with each test session. These data hence suggest that the changes in the serum concentration of gonadal hormones that occur during the menstrual cycle influence pain sensations elicited by noxious tonic cold stimulation, and show that adaptation to the cold pressor test may be sex dependent.

Key words: 17β-estradiol, progesterone, menstrual cycle, tonic cold pain
INTRODUCTION

There is considerable evidence that gonadal hormones influence pain sensitivity. Animal experiments have shown that the pregnancy induced analgesia, reported also in humans (8), is mediated by changes in circulating 17β-estradiol and progesterone that occur as a natural consequence of gestation (31), and further, gonadal hormone administration to gonadectomized female and male rats similarly increases pain threshold (10, 30). Also testosterone has been shown to modulate pain sensitivity in mammals (18, 19, 21), as well as in domestic and wild birds (15, 25). Gintzler and collaborators have demonstrated that the ovarian sex steroid antinociception is opioid-mediated and results from the activation of spinal cord kappa and delta opiate receptors (9). Consistent with these findings estrogen and progesterone receptors have been demonstrated in the spinal dorsal horn (3, 27, 43). Many of the estrogen receptor expressing neurons are opioidergic (2) and show increased opioid transcription upon 17β-estradiol administration (1). These observations provide a putative mechanism for the antinociceptive effect of estradiol, as well as for testosterone by its local aromatization to estradiol in the dorsal horn of the spinal cord (6, 14). However, in addition to opioids, the inhibitory neurotransmitter γ-aminobutyric acid (GABA) has also been shown to plays a role in pain modulation (13), and progesterone, through its neuroactive metabolites, may exert an antinociceptive effect via the GABA_A receptor complex (20).

There has been considerable interest in whether the changes in gonadal hormone levels that occur during the menstrual cycle similar to the gestation induced hormonal changes also are associated with changes in pain sensitivity, but the results of both animal and human experiments are inconsistent (17). As pointed out by Sherman and LeResche in their recent review (39), the contradictory results in the human studies on pain responses across the menstrual cycle may at least partly be explained by the difficulty in most studies to accurately
assess phase of the cycle because of the lack of hormone measurements. Additionally, as also pointed out by these and other authors (5, 39), while menstrual cycle phase reflects a particular hormone profile, significant inter- and intra-individual variations occur in the concentrations of hormones attained at the various stages; hence, the direct comparison between hormone levels and pain sensations appears to be the adequate method to examine pain/hormone relationships.

In the present study, using the cold pressor test, we assessed pain sensations once a week across the menstrual cycle in healthy, normally menstruating women. Hormone concentrations were determined from blood samples drawn after each test session and were, in addition to menstrual phases, related to pain intensity, threshold and tolerance. Adaptations that may occur across test sessions were also examined and compared with those in a control group of men. Our findings demonstrate that pain sensations induced by the cold pressor test vary across the menstrual cycle and with repeated testing and show complex relationships to gonadal hormone levels.

MATERIAL AND METHODS

Study design
Sixteen female volunteers, mean age 27 (SD = 5) years, were recruited among students at the University of Kalmar, one of which was omitted due to an anovulatory menstrual cycle (see below). For participation in the study, the following inclusion criteria were used: Age between 18 and 35 years; regular menstrual cycle (28 ± 4 days); no hormonal treatment during the past three months; and body mass index (BMI) < 30 kg/m². Exclusion criteria were use of psychotropic drugs, beta-blockers or analgetic medication (24 h before measurements); neurological disease; diabetes mellitus; history of alcohol- or drug abuse; and decreased
vascular perfusion. One of the participants was a smoker, but smoking was not allowed for a period of two hours before the test session.

A second group consisting of ten men, age 25 (SD = 4) years, was also recruited among students at the University of Kalmar. The same inclusion/exclusion criteria, when relevant, were used as described above. As in the female group, one man was a smoker.

Test sessions took place once a week during a period of 28 days, i.e. during the time span of a standardized menstrual cycle. Each session lasted about 30 min. The female participants joined the study at a random time point of their menstrual cycle, blinded for the experimenter. At the first day of the study, a diary for the following four weeks was distributed in which the days of menstrual bleeding were noted. It was the same experimenter (KS) for all the participants and test sessions.

The study was approved by the local ethical committee at Linköping University and adheres to the principles of the Declaration of Helsinki. All the subjects gave their written as well as oral consent to participate. They were informed that they could discontinue the study whenever they wanted and without giving any reason for their decision.

*Experimental test set-up*

A cold pressor test was used as stimulus source. The left hand was immersed up to the wrist in ice-chilled water (1.5 ± 0.5°C). The water tub (2.8 l) was shaken manually by the experimenter every 30 sec to avoid that the water be warmed up around the skin. The temperature in the tub was measured with a steel probe digital thermometer (VEE GEE
Scientific, Inc., Kirkland, WA, USA), and never reached above 2°C. The participants were instructed to hold their hand in the ice-water as long as possible, or to the cut-off limit, 300 seconds, was reached. As soon as the participants immersed their hand they started a computerized visual analogue scale (VAS)-rating (Pain-Test®, Archebyte, Kalmar, Sweden), a screen picture showing a line marked “no pain” at the left of the line and “worst possible pain” at the right. The participant then continually graded the perceived pain sensation by moving with their right hand the left and right arrow keys on the computer keyboard. The following outcomes were measured: (i) tolerance time (TT), (ii) activation time (AT), and (iii) highest VAS rating levels (VASmax). The definitions for the used outcomes were for TT, the time period the participant managed to keep their hand immersed in the water; for AT, the time point at which the participant reached the VAS rating level of 30 out of 100, which corresponds to the transition between mild and moderate pain in clinical practice (23, 33, 34); and for VASmax, the highest VAS rating level that occurred at a given time point during the stimulus session. The latter was determined as the shortest time across the test sessions at which VASmax was reached; hence the VAS rating was recorded at the same time point during all sessions for each subject. Taken together, these outcomes measured the pain variables tolerance, threshold and pain intensity. These recordings were followed by a post stimulus rating, performed 30 sec after the participants had withdrawn their hand. A manual 100 mm VAS-instrument marked “no pain” to the left of the line and “worst possible pain” to the right was used. The participants were asked to rate (i) the actual pain sensation at that time (VASa), and (ii) the unpleasantness of the cold pressor test (VASu) (35). The questions asked were “How painful is it now?” and “How unpleasant was the stimulus session?”. 
Laboratory analyses and definition of menstrual phases

Venous blood samples were drawn from the participating women at end of each test, and were subjected to analysis of serum hormone levels. Serum concentrations of 17β-estradiol were determined by electrochemical luminescence (medElecsys 2010, Roche, Basel, Switzerland) and s-progesterone was determined with floroimmunoassay (AutoDELFIATMProgesterone, PerkinElmer, Turku, Finland). Hormonal reference values were as follows: S-estradiol in the follicular phase between 80-590 pmol/l, during ovulation between 200-1200 pmol/l, and in the luteal phase between 130-780 pmol/l. S-progesterone in the follicular phase was < 3 nmol/l, and in the mid-luteal phase > 13 nmol/l.

The participants’ menstrual cycle length was in average case 27 days (range 24-30), as determined from the initial interview. Based on the notes in the participant’s diary about the time point of the first day of the last menstruation, complemented with the hormone values that were obtained, the test sessions were classified into the early follicular, late follicular, early luteal or late luteal phase, respectively, with preovulation values being assigned to the late follicular phase. Twelve out of the 16 women displayed this sequence during the four test sessions, but at random starting order. Because of a short menstrual cycle length, one of the participating women displayed the sequence of early follicular phase, late follicular phase, early luteal phase and again early follicular phase. Two women with long menstrual cycle length displayed early follicular phase at two consecutive sessions, and with none of the sessions occurring during the late luteal phase. Finally, one of the participating women showed an atypical hormonal profile with absence of s-progesterone increase during the late half of the menstrual cycle - a pattern associated with anovulation – and, as mentioned above, she was therefore excluded from the study. Details on hormone concentrations in the different menstrual phases to which the test sessions were assigned are shown in Table 1.
Statistical analyses
All statistical analyses were performed using MiniTab v.13, (Minitab Inc, Quality Plaza, State College, U.S.A.) and SPSS v.12.01, (SPSS Inc., Chicago, USA). Four different models were used, depending on the purpose of the analysis, which was to evaluate pain sensations in relation to (i) session-to-session effects; (ii) sex; (iii) the different menstrual phases; and (iv) the serum hormone levels.

All used analyses were built on general linear models (GLM), which gives the possibility to combine analysis of factors (categorical explanation variables such as sex) in the same way as ANOVA with analysis of covariates (continual explanation variables such as hormone levels) as in regression analysis. Furthermore, the GLM are compatible with both a balanced and unbalanced design, and, accordingly, the fact that a number of women in the present study did not do the tests in all cycle phases (see above) did not introduce any bias in the statistical analysis. The principles for how we handled explanation variables with different characteristics were as follows: (i) in comparisons between menstrual phases, the phase was used as fixed factor (i.e. as a categorical explanation variable, implying that all categories that exist or which are of interest are present in the study); (ii) because of the repeated measurement design, person was used as one factor and because person represented a sample, person was used as random factor (i.e. a categorical explanation variable from which a random sample was taken); (iii) in analysis of sex differences, person was used as nested factor within each sex (implying that the analysis assumes independence between the two sex groups, though keeping track of and matching data from each person within respective sex); (iv) in analysis of the influence of the hormone levels, these were considered as covariates (i.e. continuous explanation variables); (v) in comparisons between sessions, session was considered as a covariate (i.e. as an event at a certain time point on a continuous time scale);
(vi) in comparisons between the sexes the hormone levels of women were centered by subtracting their average hormone level; this results in an average centered hormone level of zero, a value that was also assigned to the hormone level of men, hence permitting analysis of difference in outcomes between men and women at the average hormone level of each sex; (vii) in pair-wise comparison between different categories in a fixed factor, Tukey's post hoc test was used (correcting for multiple comparison, so that a controlled simultaneous risk level is obtained for all pair-wise comparisons). Statistical significance was set at $P < 0.05$, except for the post hoc tests, in which significance, according to Turkey’s test, was set at adjusted $P < 0.05$.

RESULTS
Significant differences between the participants were seen in every test. Importantly, 9 out of the 15 women reached the 300 sec cut off limit in the cold pressor test; 2 reached the limit in all four sessions, and 2 reached the limit during three sessions. Among the men, 6 (out of 10) reached the cut off limit; 4 reached the cut off limit in all four sessions, one reached the limit during three sessions, and one reached the limit during one session. In these cases, the TT was recorded as 300 seconds. One participating woman showed to be insensitive to the cold pressor test, since her VASmax never exceeded 8 (out of 100) during the entire session, thus being an extreme outlier. Because of her insensitivity we excluded her data in the analysis of session-to-session effects and in the analysis of the hormone influence on the pain sensations.

Session-to-session effects
Because of the within-subject design of the present study, we first checked for any session-to-session effects. We found that both women and men displayed such effects. While statistically not significant, men showed a tendency towards longer AT (+5.7 sec/session), increased TT (+4.9 sec/session), and lower VASmax (-1.7 mm/session) across test sessions (Tables 2-4).
The other outcomes, VASa (+0.2 mm/session) and VASu (-0.8 mm/session) were quite stable across the test sessions (data not shown). In women, the session-to-session effects were more pronounced for AT (+18.9 sec/session) and VASmax (-5.3 mm/session), while it was of the same magnitude as in men for TT (+4.7 sec/session) (Tables 2-4). In addition, women showed decreasing VASu (-5.5 mm/session) across session, whereas VASa was rather constant (+1.3 mm/session) (data not shown). These effects were statistically significant for AT ($F_{1, 38} = 6.65; P = 0.014$), VASmax ($F_{1, 38} = 6.40; P = 0.016$, and VASu ($F_{1, 38} = 5.01; P = 0.031$). Data presented below were all corrected for these session-to-session effects.

**Sex differences**

The data revealed no statistically significant differences between women and men, although there was a tendency that men displayed longer TT (Table 2), and lower VAS ratings (Table 4), whereas AT was rather similar between sexes (Table 3).

**Relationship between menstrual phase and pain sensation**

Examination of the recorded pain sensations during the different menstrual phases showed statistically significant differences for AT ($F_{3,38} = 3.02; P = 0.042$), and post-hoc test revealed that AT was shorter during the late luteal phase compared with the late follicular phase ($P < 0.05$) (Table 2). For the other outcomes there were no significant differences between the different phases (Tables 2-4; data not shown for VASu and VASa), although there was a tendency that VASmax was higher during the late luteal phase than during the earlier phases (Table 4).
Hormone levels and pain sensations

Given that the data on pain sensations across the menstrual cycle are influenced by interindividual variations in hormone levels, we then examined the relationship between the tested outcomes and the actual hormone levels recorded at each session. This analysis showed a strong influence by the progesterone level on AT and VASmax. Thus, increasing progesterone levels at low estradiol concentrations resulted in decreasing AT ($F_{1,38} = 7.19, P = 0.011$) and increasing VASmax ($F_{1,38} = 12.27, P = 0.01$). In contrast, there were no significant relationships between the estradiol concentration by itself and any of the measured outcomes. However, analysis of the combined effects of estradiol and progesterone showed that with increasing concentrations of estradiol the pro-nociceptive effect of progesterone on VASmax was attenuated ($F_{1,38} = 5.79, P = 0.021$), and that high levels of both hormones subserved an antinociceptive effect. This relationship is illustrated graphically in Fig. 1. While a similar tendency was seen for AT ($F_{1,38} = 3.22, P = 0.08$), no specific hormone interaction patterns were found for TT, VASa and VASu.

DISCUSSION

The present study provides evidence that pain responses vary across the menstrual cycle, and that these differences are related to differences in hormone levels. Hence, the time that elapsed before the cold pressor stimulus was perceived as moderately painful was significantly reduced during the late luteal phase compared with the late follicular phase, suggesting a reduced pain threshold during the late luteal phase. The luteal phase is characterized by high progesterone levels, and, being consistent with the observed difference in AT between the late luteal and late follicular phase, analysis of the relationship between serum hormone levels and pain responses showed that high progesterone concentrations were associated with reduced AT, as well as with increasing pain intensity (VASmax). However,
the present study also demonstrates a significant interaction between estradiol and progesterone on the pain sensation. The findings show that when the levels of both hormones are considered, the increased perceived pain intensity (VASmax) that was associated with high progesterone levels was significantly reduced with increasing levels of estradiol, and, in addition, there was a tendency towards elevated pain threshold (AT) with increasing levels of both hormones. Thus, the present data suggest that gonadal hormones play complex roles in pain sensation, which may be either pro- or anti-nociceptive, depending on the hormone profile. Such complex interactions between estradiol and progesterone are consistent with observations in animal experiments (19, 22, 29).

As illustrated in Figure 1, the present analysis also indicates that high concentrations of estradiol and progesterone in fact may reduce perceived pain intensity, and hence have an anti-nociceptive effect. This would be consistent with the sex steroid dependent, gestation-associated anti-nociception that has been demonstrated in experimental animals (31), and with the pregnancy induced analgesia in humans (8). However, because few of the obtained hormone values in the present study were in the vicinity of diminishing pain intensity, this association between anti-nociception and high estradiol and progesterone levels is putative and will need further experimental support.

With respect to the role of progesterone in pain sensation, the present findings may be interpreted in two, mutually exclusive ways. One is that progesterone is pronociceptive, as suggested by the significant correlation found between the s-progesterone level and reduced activation time and increased pain intensity. This interpretation is seemingly consistent with the reduced activation time and the tendency to increased pain intensity during the late luteal phase compared to the early follicular phase. However, as shown in Table 1, the progesterone
concentration was in average somewhat higher during the early luteal phase than during the late luteal phase, yet there was no significant change in pain sensations during the early luteal phase compared with the follicular phases (Tables 3-4). Therefore, an alternate interpretation of the present data that has to be considered is that it is not the absolute hormone levels that influences pain sensations but rather their dynamic changes. While the progesterone, as well as the estradiol, concentration rises during the early luteal phase to a mid-luteal peak, the late luteal phase is characterized by falling progesterone (and estradiol) concentrations. Accordingly, it is conceivable that it is the reduced progesterone concentrations, from a high level, rather than the progesterone concentration itself that triggers the increased pain sensations during the late luteal phase. This interpretation is also consistent with the idea that progesterone subserves an inhibitory effect on neuronal excitability, as shown for example by the anesthetic effect of the progesterone metabolite pregnanolone (38). An increased pain sensation elicited by a fall in s-progesterone would be similar to the role of gonadal hormones in some cases of epilepsy (catamenial epilepsy), in which seizures are triggered by changes in hormone levels, such as during the menstrual cycle. Thus, it has been shown that progesterone reduces neuronal excitability, being anti-convulsant (37), and that the largest incidence of seizures occurs pre- and perimenstrually and are associated with the rapid decrease in progesterone during the period (4). A comparison of pain sensitivity during the mid-luteal and late luteal phases could help resolve this issue.

In addition to progesterone withdrawal as an explanation for increased pain sensitivity in the late luteal phase, tolerance development in the GABAA receptor system elicited by progesterone metabolites may also be considered. Such a mechanism has been shown for the sedative effect of pregnanolone and allopregnanolone in certain groups of women (41).
There is a large literature about pain perception across the menstrual cycle in normal healthy women, but few studies have related the findings to peripheral levels of ovarian steroids. Fillingim and co-workers (16) found that the estradiol concentration was positively correlated with thermal pain sensitivity, but not with ischemic pain sensitivity, whereas Söderberg and collaborators (40), using quantitative sensory testing, found no relationships between estradiol or progesterone plasma levels and thermal pain thresholds. Of particular relevance in relation to the present findings is the recent study by Kowalczyk and collaborators (28) in which pain sensitivity was examined using the cold pressor test. In contrast to the present findings, they report that neither estradiol nor progesterone was significantly correlated with pain threshold or tolerance. However, it is not clear from any of these previous studies whether estradiol/progesterone interactions were considered.

In addition to the study by Kowalczyk et al. (28), discussed above, two previous studies on pain sensitivity during the menstrual cycle have used cold pressor test as pain stimulus, although none of these studies measured gonadal hormone levels. One study, by Hellström and Lundberg (26), reported significantly higher pain threshold during the second phase of the menstrual cycle, whereas another study (24) demonstrated increased pain threshold during the follicular phase. The results of the latter study, however, are not directly comparable with the present work and with that of Hellström and Lundberg (26), because the subjects in that study included dysmenorrheic women, and in addition used a between-subjects design. Finally, Kowalczyk et al. (28) found no significant differences in pain threshold and tolerance as a function of menstrual cycle, but their observations were in the same direction as in the study by Hellström and Lundberg (26).
The present data obtained on the tolerance time in the cold pressor test were confounded by the fact that many participants reached the cut-off limit of 300 sec, which may explain why the TT, in contrast to AT and VASmax, showed no clear relationship with gonadal hormone levels. It is possible that permitting a longer exposure to the cold pressor test may have displayed a significant relationship, but a considerable number of participants were reported by Hellström and Lundberg (23) to have exceeded the time limit also when 420 sec were used as cut-off time. [In this context it should be noted that the average tolerance time was longer in the present study, as well as in that by Hellström and Lundberg (23), compared with other studies performed under similar conditions (32). Considering the presence of ethnic differences for the tolerance to cold pressor test (7), the sample of study objects, in both studies mainly encompassing young adult Swedes, may have played a role.] However, an alternative interpretation to the differences in outcome between TT, and AT and VASmax may be that pain tolerance, threshold and intensity are associated with different aspects of the pain perception (36), and, therefore, may be differently influenced by gonadal hormones (39).

Although men on average displayed longer tolerance time and longer pain intensity than women in the present study, these differences were not statistically significant. Also in the study by Kowalzkyk et al. (28), in which the number of participants was larger than in the present study (20 men and 21 normally menstruating women, vs. 10 and 15 in the present study) no statistically significant differences between men and women were observed, and, as discussed by these authors, this is likely due to sample size. Previous studies specifically designed to investigate gender differences in which the number of participants has been in the order of 200 have demonstrated significant sex differences in response to the cold pressor test (11, 12, 42).
Because of the large inter-individual variations in hormone levels in normally menstruating women, most studies examining the relationship of menstrual cycle phases to pain sensations use a within-subject design (see ref. 39). Since such a design involves repeated measurements in the same individual, it is of considerable interest to examine to what extent the pain responses change in response to the repeated administration of the pain stimulus. In the present study, there were strong and statistically significant session-to-session effects among the group of women, with lower reported pain intensity and increasing activation time for each session. In contrast, while similar adaptations were seen in the group of men, these effects were less pronounced. These data are consistent with those of Kowalczyk and collaborators (28), who reported that pain threshold and tolerance increased across consecutive sessions much more in normally menstruating women than in men. It was suggested that these adaptations are dependent on fluctuating levels of estradiol and progesterone, since changes in pain threshold and tolerance did not occur in a control group of women maintained on monophasic oral contraceptives (28). Taken together with these observations, the present findings are of considerable methodological interest, since they show that session sequence needs to be considered both in study design and when statistically analyzing data obtained by repeated measures across the menstrual cycle.

In summary, the present study provides evidence that pain sensation evoked by the cold pressor test, a tonic thermal stimulus, is increased during the late luteal phase in normal menstruating women. This menstrual phase difference seems to be dependent on the serum concentration of progesterone, or on changes in the s-progesterone level. However, the present data also show an interaction between progesterone and estradiol on pain sensation, with reduced pain intensity when the serum concentrations of both hormones were high. In addition, the present findings provide support for the idea that adaptation to the cold pressor
test may be sex (and hormone) dependent. While these data hence provide novel information on the role of sex steroid for pain sensitivity in the cold pressor test, further work is needed to examine if similar relationship also exist for other types of experimental pain.

GRANTS

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41. Sundström I and Bäckström T. Patients with premenstrual syndrome have decreased saccadic eye velocity compared to control subjects. *Biol Psychiatry* 44: 755-764, 1998.


LEGEND TO FIGURE

Figure 1. Graph showing the relationship between pain intensity in the cold pressor test and serum concentrations of 17β-estradiol and progesterone, as obtained from a general linear statistical model (see Materials and Methods). At low concentrations of estradiol, increasing concentrations of progesterone result in increasing VAS-rating. However, when both estradiol and progesterone increase, the pro-nociceptive effect of progesterone is attenuated. The model also predicts that high concentrations of both estradiol and progesterone will subserve an anti-nociceptive effect, as shown by the downward slope of the value plane.
Table 1. Serum concentrations of 17β-estradiol and progesterone (Mean ± SD) in 15 normally menstruating women across their menstrual phases

<table>
<thead>
<tr>
<th></th>
<th>Follicular phase</th>
<th>Luteal phase</th>
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<tbody>
<tr>
<td></td>
<td>Early</td>
<td>Late</td>
</tr>
<tr>
<td>Number of phases</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>examined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17β-estradiol, pmol/l</td>
<td>173 (76)</td>
<td>553 (371)</td>
</tr>
<tr>
<td>progesterone, nmol/l</td>
<td>1.6 (1.1)</td>
<td>2.3 (1.7)</td>
</tr>
</tbody>
</table>
Table 2. Cross table showing average tolerance time (TT; sec) in the cold pressor test across sessions (horizontal lines) and menstrual phases (vertical lines) in women, and across sessions in men (bottom horizontal line). Each value, except the unweighted mean, represents the average value for the respective cycle phase and session number in women, and average value for the respective session number in men. Unweighted mean is the average value for all session numbers in the each phase, and for all phases in each session number, respectively.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Session number</th>
<th></th>
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<th></th>
<th>Unweighted mean of all sessions</th>
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<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
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<tr>
<td>Early follicular</td>
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<td>192</td>
<td>197</td>
<td>201</td>
<td>188</td>
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<tr>
<td>Late follicular</td>
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<td>193</td>
<td>198</td>
<td>203</td>
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<tr>
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<td>183</td>
<td>188</td>
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<tr>
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<td>206</td>
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<tr>
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<td>192</td>
<td>197</td>
<td>202</td>
<td>188</td>
</tr>
<tr>
<td>Men</td>
<td>228</td>
<td>233</td>
<td>238</td>
<td>242</td>
<td>230</td>
</tr>
</tbody>
</table>
Table 3. Cross table showing the average time points (sec) at which the participants reached the VAS rating level of 30 out of 100 (AT, activation time) in the cold pressor test across sessions and menstrual phases in women and across sessions in men. For table design, see legend to Table 2.

<table>
<thead>
<tr>
<th>Phase</th>
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<th>Unweighted mean of all sessions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>2</td>
</tr>
<tr>
<td>Early follicular</td>
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<td>104</td>
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<tr>
<td>Late follicular</td>
<td>89</td>
<td>107</td>
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<tr>
<td>Early luteal</td>
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<td>Late luteal</td>
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<td>42</td>
</tr>
<tr>
<td>Unweighted mean of all phases</td>
<td>67</td>
<td>86</td>
</tr>
</tbody>
</table>

Men | 87 | 93 | 99 | 105 | 96 |

*P < 0.05 compared with late follicular phase. †P < 0.05 across sessions.
Table 4. Cross table showing average maximal pain intensity (VASmax) on a 0-100 visual analogue scale across sessions and menstrual phases in women and across sessions in men. For table design, see legend to Table 2.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Session number</th>
<th>Unweighted mean of all sessions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>2</td>
</tr>
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<td>59</td>
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<tr>
<td>Late follicular</td>
<td>70</td>
<td>65</td>
</tr>
<tr>
<td>Early luteal</td>
<td>67</td>
<td>61</td>
</tr>
<tr>
<td>Late luteal</td>
<td>79</td>
<td>73</td>
</tr>
<tr>
<td>Unweighted mean of all phases†</td>
<td>70</td>
<td>64</td>
</tr>
<tr>
<td>Men</td>
<td>58</td>
<td>56</td>
</tr>
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</table>
Figure 1. Graph showing the relationship between pain intensity in the cold pressor test and serum concentrations of 17β-estradiol and progesterone, as obtained from a general linear statistical model (see Materials and Methods). At low concentrations of estradiol, increasing concentrations of progesterone result in increasing VAS-rating. However, when both estradiol and progesterone increase, the pro-nociceptive effect of progesterone is attenuated. The model also predicts that high concentrations of both estradiol and progesterone will subserve an anti-nociceptive effect, as shown by the downward slope of the value plane.