Endogenous pain inhibition during menstrual cycle in migraine

M. Teepker¹, M. Kunz², M. Peters²,³, B. Kundermann⁴, K. Schepelmann¹,⁵, S. Lautenbacher²

¹ Department of Neurology, Philipps-University of Marburg, Germany
² Department of Physiological Psychology, University of Bamberg, Germany
³ Department of Child and Adolescent Psychiatry, Philipps-University of Marburg, Germany
⁴ Department of Psychiatry and Psychotherapy, Philipps-University of Marburg, Germany
⁵ Department of Neurology, Schlei-Klinikum Schleswig MLK, Germany

Abstract

Background: Migraine is a common headache disorder that can vary menstrually in women and has been linked to an impairment of endogenous pain inhibitory systems. One of these endogenous pain inhibitory systems, namely conditioned pain modulation (CPM; formerly diffuse noxious inhibitory controls-like), has been shown to be affected by the menstrual cycle. The aim of this study was to examine CPM over the menstrual cycle in migraineurs and healthy controls.

Methods: Twenty healthy women and 32 female migraineurs were examined on days 1, 4, 14 and 22 of the menstrual cycle. Detection and pain thresholds for electrocutaneous stimuli were first assessed at baseline. Second, tonic heat stimuli were applied concurrently to the electrical stimuli, and the difference in electrical thresholds to baseline were analysed as indicating CPM inhibition.

Results: Migraineurs revealed higher detection thresholds than the control group but similar pain thresholds for electrotaneous stimuli were first assessed at baseline. Second, tonic heat stimuli were applied concurrently to the electrical stimuli, and the difference in electrical thresholds to baseline were analysed as indicating CPM inhibition.

Conclusions: Our findings suggest that CPM inhibition is not altered in female migraineurs; thus, it is questionable whether CPM really plays a role in the development of migraine or whether migraine leads to a dysfunctional CPM inhibition.

1. Introduction

The finding that one painful stimulus can inhibit the perception of a second one has been called conditioned pain modulation (CPM) (Yarnitsky et al., 2010). Deficiencies in CPM inhibition indicate an impairment of the descending antinociceptive pathways and are acclaimed to be risk factors for the development of chronic pain [e.g., chronic tension-type headache (CTTH); Pielsticker et al., 2005]. The physiological basis for CPM is supposed to lie mainly in the diffuse noxious inhibitory controls, which control the inhibition of nociceptive wide dynamic range neurons in the spinal and trigeminal horn elicited by noxious stimuli outside their receptive fields. The supraspinal components involved in CPM are assumed to be subnucleus reticularis dorsalis in the caudal medulla (Hu, 1990; Bouhassira et al., 1992), orbitofrontal cortex (Piché et al., 2009; Moont et al., 2011), amygdala (Piché et al., 2009; Moont et al., 2011), anterior cingulate cortex (Piché et al., 2009; Sprenger et al., 2011), primary somatosensory cortex (Piché et al., 2009) and the supplementary motor area (Piché et al., 2009).

Although the pathophysiology of migraine is not completely understood, migraine is thought to be a...
neurovascular disorder (Goadsby et al., 2002) with impaired descending antinoceptive pathways (Welch et al., 2001). In line with this, Sandrini et al. (2006) found CPM dysfunction in patients suffering from migraine. Likewise, Tommaso et al. (2007) also found CPM dysfunction in chronic migraineurs; however, patients with more episodic migraine without aura did not show this deficit. Interestingly, Perrotta et al. (2010) also found no CPM dysfunction in patients with episodic migraine compared with healthy controls (CPM deficits were apparent only in patients with medication-overuse headache). These contradictory findings suggest that CPM deficiency seems to be of still unclear relevance in migraineurs, with positive results seemingly confined to chronic migraine. It is also far from clear whether this CPM deficit precedes, succeeds or parallels the migraine attacks, which can be determined only in longitudinal studies.

Migraine can vary menstrually, and some women report onset of migraine either some days before or some days after the start of the menstrual phase (Silberstein and Merriam, 1993; Johannes et al., 1995; MacGregor et al., 2006), which might be due to the decrease of oestrogen at the end of the menstrual cycle (MacGregor et al., 2006). Interestingly, the menstrual cycle has not only been shown to impact the occurrence of migraine but also to influence CPM inhibition, with more pronounced CPM effects during the ovulatory phase in healthy women (Tousignant-Laflamme and Marchand, 2009; Rezaei et al., 2012).

The aim of the present study was to investigate CPM inhibition in migraineurs using a longitudinal design, which allowed for determining whether impairment in CPM is associated, either constantly or transitorily, with migraine. This design also allowed for testing on the stability of CPM functions. For that purpose, we studied CPM inhibition in female migraineurs and healthy controls during different phases of the menstrual cycle by means of an established CPM paradigm already used for the study of tension-type headache (Pielsticker et al., 2005).

2. Methods

2.1 Subjects

Participants were recruited via wall posters and advertisements in local newspapers. They were paid for attendance and gave written informed consent before participating in the study. The experimental protocol was approved by the ethics committee of the medical school of the University of Marburg. All women who responded were interviewed via telephone regarding the inclusion and exclusion criteria. The exclusion criteria for all subjects were pregnancy, hypertension, headache diseases other than migraine, other acute and chronic pains, endocrine disorders (e.g., of the thyroid gland), gynaecological diseases, psychiatric disorders, peripheral and central neuropathies as well as dermatosis at the site of the pain stimulation, intake of drugs for the prophylactic treatment of pain syndromes or migraine, and the regular intake of analgesics. Potential candidates for participations were invited and thoroughly examined by a headache-specialized neurologist and a psychologist. Migraineurs had to suffer from migraine according to the guidelines of the International Headache Society (Headache Classification Committee of the International Headache Society, 2004). They had to answer a questionnaire in order to evaluate typical migraine symptoms such as headache intensity, headache frequency, headache duration, disease duration and autonomic attendant symptoms. Furthermore, migraineurs had to keep a headache diary for at least 2 months in order to indicate headache attacks and pain intensity, including questions for menstrual parameters (i.e., beginning and end of menstrual bleeding as well as the duration of menstrual cycles). The onset of menstrual bleeding was defined as the first day of menstrual cycle. Menstrual cycles had to be regular (28 ± 1 day) in all subjects. Healthy volunteers reported also about this in a questionnaire and a diary.

According to the headache diary of the migraineurs, 2.28 ± 0.99 headache bouts occurred per month (minimal number: 1 attack, maximal number: 5 attacks). Analgesics were only allowed if a migraine attack appeared shortly before or during the examinations. The analgesics permitted included mainly short-lasting non-steroidal anti-inflammatory drugs or triptans. Out of the 32 migraineurs, 22 took oral contraceptives (OC) and 10 did not. None of the healthy control women took OC. To screen for psychiatric disorders, especially depression and anxiety disorders that occur often in association with migraine, the ‘Mini-
DIPS’ (the German version of the Interview of Mental Disorders short version handbook (Margraf, 1994) was used. In total, we included 20 healthy and pain-free women in the control group (mean age = 27.1 ± 6.6 years) as well as 32 female migraineurs (mean age = 28.9 ± 8.8 years). Among the migraineurs, eight patients had a migraine with aura [with a menstrual association in three subjects (9.4%) and no association in five subjects (15.6%)]. Twenty-four subjects suffered from a migraine without aura that occurred with a menstrual association in 14 women (43.8%) and without a menstrual dependency in four (12.5%) cases. Six (17.6%) women suffered from a menstrual migraine and described no aura symptoms.

2.2 General procedure

After the initial screening for inclusion and exclusion criteria, the experimental investigations (all run by two female investigators) took place in a sound-attenuated laboratory of the University of Marburg and lasted approximately 3 h each. Time of day for investigation was kept constant for each single subject over the four sessions. The sessions were run on days 1, 4, 14 and 22 (±1 day) of the menstrual cycle for all subjects included in this study. Sessions started with a careful familiarization with the sensory tests. Thereafter, sessions consisted of two parts.

In the first part, participants took part in quantitative sensory testing (QST), including assessment of a number of thresholds: warm and cold threshold, heat- and cold-pain thresholds, pressure-pain thresholds as well as detection and pain thresholds for electrical current. We previously reported on the results of the QST (Teepker et al., 2010, 2011) and do not refer to these results anymore in the present report. In the second part, a well-established CPM paradigm was used to study CPM inhibition (Lautenbacher and Rollman, 1997; Pielsticker et al., 2005).

2.3 Assessment of CPM effects – procedure

First, assessment of heat-pain thresholds became necessary to tailor the conditioning stimulus of the CPM paradigm to individual pain sensitivity. Subjects were instructed to adjust a slightly painful temperature at a Peltier thermode (surface area: 6 cm²) of a computer-controlled thermal stimulator (Thermal Sensory Analyzer TSA-2001, Medoc Ltd, Israel) by pressing either heating or cooling buttons. Constant pressing of the buttons produced rates of change of 0.7 °C/s. For safety reasons, the maximal temperature was set to 52 °C. Twenty electrical stimuli per experimental block served as test stimuli and were concurrently applied by means of a electrostimulator (Erich Jäger GmbH & Co. KG, Germany) with a maximal intensity of 10 mA to the right volar forearm (see section 3.2). Stimuli consisted of a train of 15 monophasic square wave-pulses; further parameters were: pulse duration of 4 ms, stimulus onset asynchrony of 10 ms, and duration of each electrical stimulus of 144 ms. For stimulation, two bipolar electrodes with a surface area of 0.3 cm² covered with a special cream (Abralyt 2000, FMS Falk Minow Services, Germany) were attached after skin preparation 2 cm away from each other, slightly to the left and to the right of the centre of the forearm.

2.3.1 Conditioning stimuli

Tonic thermal stimulation consisted of a series of pulses at a constant frequency of 30 pulses/min, with amplitude of 1.5 °C (Lautenbacher et al., 1995). In the ‘painful stimulation’ condition, the pulses were tailored to have a peak temperature of 1 °C above pain threshold, and in the ‘not painful condition’ of 0.3 °C below pain threshold. This approach allowed for comparing the conditioning effects of a still tolerable tonic heat pain (‘painful’) with the conditioning effects of a strong but non-painful tonic heat stimulus (‘not painful’). For ‘no thermal stimulation’, the thermode was kept in place and temperature remained at the baseline level of 35 °C. After each fifth test stimulus (electrical current), the conditioning thermal stimulus had to be estimated by the participants as ‘1’ (no sensation), ‘2’ (slight sensation), ‘3’ (moderate sensation), ‘4’ (strong sensation), ‘5’ (slight pain) or ‘6’ (moderate pain).

2.3.2 Test stimuli

Concurrently to conditioning stimulation, 20 electrocutaneous test stimuli were applied in intervals of 10 s using a double staircase procedure with two staircases, one for assessing the electrical detection threshold and one for assessing the electrical pain threshold. Participants had to estimate the intensity of each electrical stimulus on a scale of 1 = ‘no sensation’, 2 = ‘not painful sensation’ or 3 = ‘painful sensation’. According to this rating, the intensity of the electrocutaneous stimuli was modified as follows: (i) staircase detection threshold: rating (1) = ‘no sensation’ led to an increase of 0.15 mA, rating (2) = ‘not painful sensation’ or rating (3) = ‘painful sensation’ led to a decrease of 0.15 mA; (ii) staircase pain threshold: rating (1) = ‘no sensation’ or rating (2) = ‘not painful sensation’ led to an increase of 0.3 mA, rating (3) = ‘painful sensation’ led to a decrease of...
0.3 mA. The starting intensity of each staircase was adopted from the detection and pain thresholds for electrical currents in part 1 of the sessions. The investigator signalled each stimulus verbally.

### 2.4 Statistics

Descriptive data are given as mean ± standard deviation (SD). Physical units were (°C) for heat-pain thresholds and (mA) for electrical thresholds.

Using Statistical Package for the Social Sciences 20.0 (IBM Corp., Armonk, NY) for windows, analyses of variance (ANOVAs) with repeated measurements were conducted with the between-subjects factor ‘group’ (healthy controls, migraineurs) and the within-subject factors ‘day of testing’ (days 1, 4, 14 and 22) and, if applicable, ‘stimulation’ (no thermal stimulation, not painful stimulation and painful stimulation). Adjusting degrees of freedom (df) by use of Greenhouse–Geisser correction became necessary if sphericity could not be assumed. In case of significant ANOVA results, post hoc Bonferroni tests were used. Alpha was set to 0.05 throughout.

### 3. Results

#### 3.1 Migraine attacks

Migraineurs were asked for occurrence and intensity of migraine attacks 24 h before testing and occurrence of migraine during the session (Table 1 gives percentage of migraineurs who reported about migraine attacks). Nine women reported migraine attacks prior to day 1; 14 prior to day 4; one prior to day 14; and three prior to day 22. During the experiments, three participants complained of migraine on day 1, six on day 4 and two on day 22. On day 14, no one suffered from migraine headaches. In summary, we observed in our sample of migraineurs the usual increase in prevalence of migraine attacks at the beginning of the menstrual cycle during menstruation.

#### 3.2 CPM effects

##### 3.2.1 Intensity of conditioning stimulation

The conditioning stimuli were tailored to the individual heat-pain thresholds, the descriptive statistics of which are shown in Table 2a. ANOVA with repeated measurements revealed significance for the within-subject factor ‘day of testing’ (F = 3.119; df = 2.477/123.870; p = 0.037). Post hoc analysis demonstrated a significant difference between thresholds on days 22 and 4 (day 22 > day 4; p < 0.01), whereas all other pairwise comparisons remained insignificant. Furthermore, ANOVA revealed no significant interaction effect on the ‘day of testing’ × ‘group’ (F = 0.267; df = 0.267/123.870; p = 0.811). Most importantly, no main effect could be detected for the differences in heat-pain thresholds between the control and migraine group (F = 0.577; df = 1/50; p = 0.451). The latter finding suggests that the physical intensity of the conditioning stimuli was similar in the two groups.

The ratings of the conditioning heat stimulation are presented in Table 2b. ANOVA with repeated measures showed that there was no significant main effect which is shown in Table 2b. ANOVA with repeated measurements revealed significance for the within-subject factor ‘day of testing’ (F = 3.119; df = 2.477/123.870; p = 0.037). Post hoc analysis demonstrated a significant difference between thresholds on days 22 and 4 (day 22 > day 4; p < 0.01), whereas all other pairwise comparisons remained insignificant. Furthermore, ANOVA revealed no significant interaction effect on the ‘day of testing’ × ‘group’ (F = 0.267; df = 0.267/123.870; p = 0.811). Most importantly, no main effect could be detected for the differences in heat-pain thresholds between the control and migraine group (F = 0.577; df = 1/50; p = 0.451). The latter finding suggests that the physical intensity of the conditioning stimuli was similar in the two groups.

#### Table 1

<table>
<thead>
<tr>
<th>Pain intensity</th>
<th>Day 1 (%)</th>
<th>Day 4 (%)</th>
<th>Day 14 (%)</th>
<th>Day 22 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h prior to testing</td>
<td>Mild 16</td>
<td>22</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Moderate 6</td>
<td>13</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Severe 6</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total during testing</td>
<td>28</td>
<td>44</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Total during testing session</td>
<td>9</td>
<td>19</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

#### Table 2

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Heat-pain threshold (used as reference values for the conditioning stimulus in the CPM paradigm)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.13 ± 0.22</td>
</tr>
<tr>
<td>4</td>
<td>1.13 ± 0.24</td>
</tr>
<tr>
<td>14</td>
<td>1.40 ± 0.60</td>
</tr>
<tr>
<td>22</td>
<td>1.24 ± 0.36</td>
</tr>
<tr>
<td>Not painful stimulation</td>
<td>4.03 ± 0.79</td>
</tr>
<tr>
<td>(35 °C)</td>
<td>3.80 ± 0.64</td>
</tr>
<tr>
<td>14</td>
<td>3.80 ± 0.73</td>
</tr>
<tr>
<td>22</td>
<td>3.81 ± 0.72</td>
</tr>
<tr>
<td>b) Ratings of the conditioning stimuli</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.76 ± 0.72</td>
</tr>
<tr>
<td>Stimulation (1 °C) above pain threshold)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4.86 ± 0.79</td>
</tr>
</tbody>
</table>
Table 3. Results of ANOVA for repeated measurements for the influence of group (‘G’: healthy volunteers vs. migraineurs), day of testing (‘D’: day 1 vs. 4 vs. 14 vs. 22) and stimulation (‘S’: no thermal stimulation vs. not painful stimulation vs. painful stimulation) on (a) the subjective evaluation of the conditioning heat stimulus, (b) the electrical pain thresholds and (c) the electrical detection thresholds.

<table>
<thead>
<tr>
<th>Factors</th>
<th>df</th>
<th>F</th>
<th>p</th>
<th>Etat^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Subjective evaluation of the conditioning heat stimulus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group (G)</td>
<td>1/50</td>
<td>0.395</td>
<td>0.532</td>
<td>0.008</td>
</tr>
<tr>
<td>Day of testing (D)</td>
<td>1.994.8</td>
<td>7.791</td>
<td>&lt;0.001</td>
<td>0.135</td>
</tr>
<tr>
<td>Stimulation (S)</td>
<td>2/100</td>
<td>680.370</td>
<td>&lt;0.001</td>
<td>0.932</td>
</tr>
<tr>
<td>G × D</td>
<td>1.994.8</td>
<td>0.865</td>
<td>0.419</td>
<td>0.017</td>
</tr>
<tr>
<td>G × S</td>
<td>2/100</td>
<td>0.357</td>
<td>0.701</td>
<td>0.007</td>
</tr>
<tr>
<td>D × S</td>
<td>3.7/186.5</td>
<td>4.315</td>
<td>0.003</td>
<td>0.079</td>
</tr>
<tr>
<td>G × D × S</td>
<td>3.7/186.5</td>
<td>3.357</td>
<td>0.013</td>
<td>0.63</td>
</tr>
<tr>
<td>(b) Electrical pain thresholds (test stimuli)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group (G)</td>
<td>1/50</td>
<td>1.443</td>
<td>0.235</td>
<td>0.028</td>
</tr>
<tr>
<td>Day of testing (D)</td>
<td>2.263/113.170</td>
<td>5.364</td>
<td>&lt;0.001</td>
<td>0.097</td>
</tr>
<tr>
<td>Stimulation (S)</td>
<td>1.625/81.270</td>
<td>51.207</td>
<td>&lt;0.001</td>
<td>0.506</td>
</tr>
<tr>
<td>G × D</td>
<td>2.263/113.170</td>
<td>0.037</td>
<td>0.990</td>
<td>0.001</td>
</tr>
<tr>
<td>G × S</td>
<td>1.625/81.270</td>
<td>1.301</td>
<td>0.277</td>
<td>0.025</td>
</tr>
<tr>
<td>D × S</td>
<td>4.770/238.504</td>
<td>1.100</td>
<td>0.360</td>
<td>0.022</td>
</tr>
<tr>
<td>G × D × S</td>
<td>4.770/238.504</td>
<td>1.549</td>
<td>0.162</td>
<td>0.030</td>
</tr>
<tr>
<td>(c) Electrical detection thresholds (test stimuli)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group (G)</td>
<td>1/50</td>
<td>7.427</td>
<td>0.009</td>
<td>0.129</td>
</tr>
<tr>
<td>Day of testing (D)</td>
<td>3/150</td>
<td>3.087</td>
<td>0.029</td>
<td>0.058</td>
</tr>
<tr>
<td>Stimulation (S)</td>
<td>2/100</td>
<td>11.732</td>
<td>&lt;0.001</td>
<td>0.190</td>
</tr>
<tr>
<td>G × D</td>
<td>3/150</td>
<td>0.039</td>
<td>0.990</td>
<td>0.001</td>
</tr>
<tr>
<td>G × S</td>
<td>2/100</td>
<td>0.832</td>
<td>0.438</td>
<td>0.016</td>
</tr>
<tr>
<td>D × S</td>
<td>4.504/225.218</td>
<td>2.643</td>
<td>0.029</td>
<td>0.05</td>
</tr>
<tr>
<td>G × D × S</td>
<td>6/300</td>
<td>0.205</td>
<td>0.975</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Significant effects are marked in bold. ANOVA, analysis of variance.

of the factor ‘group’ (see Table 3a). This is evidence – this time based on the subjective experience – that we succeeded in applying comparable intensities of the conditioning stimulus in the two groups of participants. Potential differences in CPM effects between groups can therefore be interpreted with great confidence as changes in inhibitory processes. Interestingly, subjective ratings varied significantly over time (‘D’; see Table 3a), with highest ratings on days 14 and 22, which both differed significantly (p < 0.01) from day 4, as well as with significant higher ratings on day 14 compared with day 1 (p < 0.05). Furthermore, there was a significant main effect of the factor ‘stimulation’ (see Table 3a). Detailed post hoc analysis showed not surprisingly that the ‘not painful stimulation’ was rated to be stronger than ‘no stimulation’ (p < 0.001), ‘painful stimulation’ stronger than ‘no stimulation’ (p < 0.001) or ‘not painful stimulation’ (p < 0.001). As a secondary result, we obtained significant interaction effects regarding ‘day of testing’ × ‘stimulation’ as well as ‘day of testing’ × ‘stimulation’ × ‘group’ (see Table 3a). This means that the differences between the ratings for three stimulation intensities were modified by menstrual cycle, but this effect itself is dependent on the factor ‘group’.

### 3.2.2 Effects on the electrical pain thresholds

Fig. 1A and B show the results of the inhibitory effects of the conditioning stimulus on the electrical pain thresholds. An ANOVA with repeated measurements demonstrated a statistically significant main effect of the factor ‘stimulation’ (see Table 3b), indicating that CPM effects were present. According to the post hoc analyses, pain thresholds under conditions of painful stimulation were significantly increased compared with the other conditions (both p-values < 0.001) and pain thresholds under conditions of ‘not painful stimulation’ were significantly higher than without concurrent thermal stimulation (p < 0.001). Accordingly, the CPM effects appeared to be dose-dependent or, in other words, the stronger the conditioning stimulus was, the bigger the increase was of the electrical pain threshold.

The ANOVA failed to demonstrate a main ‘group’ effect (see Table 3b), suggesting similar pain sensitivity for electrical current in the two groups. Furthermore, we observed a significant main effect for ‘time’ (‘D’, see Table 3b), i.e., the electrical pain thresholds substantially varied during menstrual cycle. Post hoc Bonferroni tests revealed significant lower thresholds on day 1 compared with day 4 (p < 0.01) and to day 14 (p < 0.001), whereas the other comparisons remained statistically insignificant.

Finally, the interaction effects G × S, D × S and G × D × S were not significant (see Table 3b). This lack of significant interactions, including the group factor, is most remarkable for the present study. It suggests that the CPM results did not appear to be differentially affected by the diagnostic group or, in other words, CPM did not differ between migraineurs and healthy control subjects.

The insignificant interaction between ‘day of measurement’ and ‘stimulation’ interestingly indicates that the CPM effects did not vary over the menstrual cycle. Because some of the migraineurs took OC, which might have affected the menstrual cycle, the analysis was repeated only in the healthy women free of OC with the same result (df = 3.303/62.765; F = 1.357; p = 0.238; Greenhouse–Geisser correction).

### 3.2.3 Effects on the electrical detection thresholds

Fig. 1C and D show the effects on the detection thresholds in the CPM paradigm. A significant main
effect for the factor ‘stimulation’ (see Table 3c) could be observed, suggesting an inhibitory effect on the detection thresholds for electrical current. Post hoc analyses showed that detection thresholds under conditions of ‘painful stimulation’ was significantly increased ($p = 0.021$) in comparison to the ‘no thermal stimulation’ condition and in tendency ($p = 0.054$), when compared with the ‘not painful stimulation’ condition. The inhibitory effects existed in both groups as the interaction ‘group’ × ‘stimulation’ was not significant (see Table 3c). Furthermore, there were higher electrical detection thresholds in the migraineurs compared with the control group (see Table 3c), indicating reduced sensitivity in the patients for non-painful currents. A main effect of ‘day of testing’ (see Table 3c) was mainly based on a nearly significant increase of detection threshold on day 4 compared with day 14. Moreover, the inhibitory effect on the detection thresholds appeared to be modified by the day of testing during menstrual cycle. This was statistically indicated by a $D \times S$ interaction (see Table 3c). This means that we

![Figure 1](image-url)
observed a particular increase of detection thresholds under ‘painful stimulation’ compared with the other conditions on day 4 (see Fig. 1C and D). Since some of the migraineurs took OC, which might have affected the menstrual cycle, the analysis was repeated only in the healthy women free of OC. The interaction of D × S became yet insignificant in this sample (df = 6/114; F = 1.96; p = 0.08). All other interaction effects (G × S, G × D and G × D × S) were already nonsignificant (see Table 3c), suggesting that the menstrual variations of the inhibitory effects did not differ between the two groups tested.

4. Discussion

We hypothesized that an impairment of endogenous pain inhibitory systems plays an important role in the pathophysiology of migraine. On the background that this dysfunction might be demonstrated using a CPM paradigm and vary over the menstrual cycle along with headache symptoms, we investigated migraineurs and healthy subjects during different phases of the menstrual cycle on days 1, 4, 14 and 22. We were able to demonstrate clear CPM effects on pain thresholds as well as inhibitory effects on detection thresholds in migraineurs when conditioning heat stimuli were applied. Most interestingly – and contradictory to our expectation – these effects did not differ from those of the control group.

4.1 CPM in migraine

Accordingly, the major finding of the present study was a constant normality of CPM inhibition over the menstrual cycle in female migraineurs. The discrepancy between our and earlier findings in this respect will be detailed by comparing studies. Sandrini et al. (2006) found that both migraineurs and patients with CTTH showed no inhibition but instead facilitation of the RIII reflex with parallel changes in subjective pain ratings during the cold pressor test (CPT). A reason for these conflicting findings might be the different methods used. Whereas Sandrini et al. (2006) investigated CPM effects on the RIII reflex and pain ratings; we used pain and detection thresholds. Furthermore, both the conditioning (cold water vs. heat via thermode) and the test stimuli (brief electrical pulses vs. extended electrical trains) differed. A recent review of the variations in CPM methodology and their effects highlighted the critical relevance of techniques and methods (Nir et al., 2011). Furthermore, Sandrini et al. (2006) included women and men, and their participants (36 ± 12 years) were also older than in our study (28.1 ± 7.6 years), which has likely also affected the findings (Larivière et al., 2007).

The studies of de Tommaso et al. (2007) and Perrotta et al. (2010) did not contradict our findings as unequivocally as those of Sandrini et al. (2006). In both of these studies, no impairment of CPM inhibition was found in patients with episodic migraine. Interestingly, the CPM paradigms used in both studies were also quite different from ours. The group of de Tommaso et al. (2007) investigated CPM function by capsaicin-induced modifications of the blink reflex, which is an unusual methodological approach to CPM, and here CPM inhibition appeared insufficient only in the patients with chronic migraine but not in those with episodic migraine. Perrotta et al. (2010) used a very similar method as Sandrini et al. (2006) and tested the nociceptive withdrawal reflex and pain ratings before, during and after CPM activation by the CPT in patients suffering from a medication-overuse headache, episodic migraine and in healthy controls. CPM inhibition was not deficient in patients with episodic migraine but only in patients with medication-overuse headache.

To summarize, it is yet not clear whether CPM deficiency can be verified in all forms of migraine by any CPM paradigms. It may well be that this is true only for the chronic forms of migraine and when studying the nociceptive reflex measures, whereas episodic forms appear spared especially when testing psychophysical parameters. Rehberg et al. (2012) recently provided convincing data that point to a dissociation between CPM effects on pain and on nociception, after having compared nociceptive reflexes and pain ratings. Future studies are necessary to further clarify this issue; however, it seems that CPM dysfunctions might not be present in all forms of migraine (more in chronic and less in episodic forms of migraine) and not in all forms of headache (maybe more in chronic tension-type headache compared with migraine (Pielsticker et al., 2005)).

4.2 CPM and menstrual variation

There were group-independent inhibitory effects on the electrical detection thresholds that varied menstrually. However, we did not observe significant changes in CPM effects on pain thresholds over the menstrual cycle in both migraineurs and healthy controls. Because some of the migraineurs took OC, which might have affected the menstrual cycle, we repeated these analyses in healthy women free of OC and found that the menstrual cycle neither affected CPM effects on pain nor on detection thresholds.
These findings are in contrast to the findings of Tousignant-Laflamme and Marchand, (2009) and Rezaii et al. (2012). Tousignant-Laflamme & Marchand (2009) demonstrated that inhibitory but not excitatory pain mechanisms varied over the menstrual cycle. The authors investigated 32 healthy women during three phases of the menstrual cycle using CPT as conditioning stimulus and tonic heat-pain stimulation as test stimulus. In this study, women had greater CPM effects in the ovulatory phase; however, the impact of the menstrual cycle on CPM was not strong, as indicated by a moderate effect size. In a very recent study, Rezaii et al. (2012) described significant CPM effects in the ovulatory phase as well. They used mechanical pressure as test stimulus and CPT for conditioning stimulation. Although they found a menstrual variation in CPM effects, there was no correlation between CPM and the serum levels of sex hormones.

It is well known and has already been mentioned earlier in this text that the results of experimentally induced CPM effects depend strongly on the paradigm used (for review, see Pud et al., 2009). This might explain why Tousignant-Laflamme and Marchand (2009) and Rezaii et al. (2012) found menstrual-dependent CPM effects and we did not. The former authors both used a similar paradigm with CPT for conditioning stimulation, which also leads to strong autonomic reactions, and pressure as test stimulus, and this differs from ours. Furthermore, participants were tested on three different days only of the menstrual cycle. We evaluated CPM effects over four different days of the menstrual cycle, which favours an adequate menstrual data sampling and sufficient statistical power. Thus, the impact that the menstrual cycle has on CPM inhibition seems to be small and might depend on the methods used.

4.3 CPM and the intensity of the conditioning stimulus

We could demonstrate a dose-dependent CPM effect of the conditioning stimuli (‘painful stimulation’ > ‘not painful stimulation’ > ‘no stimulation’) on the electrical pain thresholds, which is in line with the results of Fujii et al. (2006) or Lautenbacher et al. (2008). However, other studies could not confirm this observation (Lautenbacher and Rollman, 1997; Pielsticker et al., 2005; Kunz et al., 2006). Thus, there are contradictory findings regarding the relationship between the strength of the conditioning stimulus and the extent of the CPM effects (for review, see Pud et al., 2009). Probably, the noxious intensity of the conditioning stimulus is more important than its subjective experience in determining the effect size of CPM inhibition.

4.4 Pain and somatosensory sensitivity in general

The migraine group displayed elevated detection thresholds for electrical current but unchanged pain thresholds for electrical current and heat at a non-cranial site, indicating that there is no general hypersensitivity evident in migraine. In recent studies, detection thresholds for vibrotactile stimuli or electrical current in migraineurs did not differ from those in healthy controls (Gierse-Plogmeier et al., 2009; Nguyen et al., 2013). Furthermore, also in an earlier study of the authors (Teepker et al., 2011), no abnormalities in detection thresholds for warmth, cold and electrical current were found in migraineurs. Thus, although we found elevated detection thresholds in migraineurs in the present study, most of the evidence clearly speak against a general hypoesthesia.

The difference between pain thresholds across the menstrual cycle might be explained either by pain hyper-responsiveness in the menstrual phase (matching the increased vulnerability for clinical pain during this epoch) or by adaptation to pain stimulation starting in the first sessions during menstruation and ending later in the ovulatory or luteal phase.

4.5 Limitations of our study

A limitation of this study is the fact that most women in the migraine group used OCs (that alter hormonal cyclic fluctuations), whereas none of the pain-free women did, with both groups being of small size. Because our study design was very extensive, larger study groups were out of reach. Furthermore, menstrual cycle phases were determined by use of diaries and questionnaires but not by hormonal assays. In our earlier study (Teepker et al., 2010), the relationship between the saliva concentrations of sexual hormones (oestrogen, testosterone) and psychophysical parameters was too weak to warrant a second try. Due to our interest in potential menstrual variations, we did not test men. Moreover, the sessions over the menstrual cycle were always run in the same sequence (days 1, 4, 14 and 22), instead of using randomized starting points, which may have left order effects uncontrolled. This was carried out to avoid a further source of variations in the small samples. Lastly, the staircase method used to determine the detection and pain thresholds may be biased.
by individual response criteria; the resulting thresholds might therefore appear decreased if the response criterion is liberal (more false alarms) and increased if the criteria is conservative (more false rejections) (Yang et al., 1985). However, this disadvantage shares the staircase method with others threshold protocols and bias-free methods are not available with limited numbers of stimuli.

Summary

Migraineurs revealed no evidence for an impairment of endogenous pain inhibitory systems in a CPM paradigm. Furthermore, CPM-like effects on pain thresholds did not vary during menstrual cycle in our study.

Declarations

Original manuscript

This manuscript contains original unpublished work and is not being submitted for publication elsewhere at the same time.

Author contributions

All authors have participated sufficiently in the manuscript. They discussed the results and commented on the manuscript.

References


