Does pain sensitivity change by migraine phase? A blinded longitudinal study.

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Abstract

Objective: Studies suggest that pain thresholds may be altered before and during migraine headaches, but it is still debated if a central or peripheral dysfunction is responsible for the onset of pain in migraine. The present blinded longitudinal study explores alterations in thermal pain thresholds and suprathreshold heat pain scores before, during, and after headache.

Methods: We measured pain thresholds to cold and heat, and pain scores to 30 seconds of suprathreshold heat four times in 49 migraineurs and once in 31 controls. Sessions in migraineurs were categorized by migraine diaries as interictal, preictal (≤ one day before attack), ictal or postictal (≤ one day after attack).

Results: Trigeminal cold pain thresholds were decreased ($p = 0.014$) and pain scores increased ($p = 0.031$) in the ictal compared to interictal phase. Initial pain scores were decreased ($p < 0.029$), and the temporal profile showed less adaptation ($p < 0.020$) in the preictal compared to interictal phase. Hand cold pain thresholds were decreased in interictal migraineurs compared to controls ($p < 0.019$).

Conclusion: Preictal heat hypoalgesia and reduced adaptation was followed by ictal trigeminal cold suballodynia and heat hyperalgesia. Our results support that cyclic alterations of pain perception occur late in the prodromal phase before headache. Further longitudinal investigation of how pain physiology change within the migraine cycle is important to gain a more complete understanding of the pathogenic mechanisms behind the migraine attack.

Key Words

Headache; migraine cycle; preictal; premonitory; allodynia; hyperalgesia.

Introduction

Altered pain perception may be of importance for migraine pathophysiology. Several studies have shown decreased experimental pain thresholds and increased pain scores in migraineurs in the headache-free interval (interictal phase) compared to healthy controls (1-13). About sixty percent of migraineurs report cutaneous allodynia during headache (ictal phase) (14-17). This is comparable to the proportion with headache-related allodynia found in an experimental study (18).

Various symptoms may precede the headache, e.g. yawning, mood change, lethargy, neck symptoms and light sensitivity (19-23). However, little is known about the central mechanisms and sequence of events that initiates these warning/premonitory symptoms. Several symptoms may also outlast the headache (postdromal symptoms) (19, 22, 24, 25). The premonitory and postdromal symptoms, as well as imaging (26-28) and neurophysiological (29-38) findings indicate that migraine is driven by cyclic central nervous system alterations that precedes and outlasts the ictal phase.

Several studies have shown increased responses to experimental pain during the ictal phase compared to the interictal phase (11, 39-43). Although the alteration in pain perception is most pronounced during migraine attacks, subtler changes may be present before and after the headache (preictal and postictal phase, respectively). Few have investigated pain-related physiological changes across migraine phases. A longitudinal study demonstrated decreased thermal pain thresholds preictally compared to interictally (36). An association between heat

pain thresholds and hours to the next attack (10), and an association between activation in the spinal trigeminal nuclei by nociceptive stimuli and the time to the next attack (44), have also been reported. Exploring pain perception in the preictal and postictal phases could contribute to a better understanding of the pathophysiology (45).

Experimental tonic pain may resemble clinical pain better than pain thresholds (46), and the temporal profile may reflect both peripheral and central mechanisms (47, 48). Furthermore, in order to elucidate migraine mechanisms, intraindividual changes to tonic painful stimulation during the different migraine phases may be more relevant than comparing migraineurs in the interictal phase to healthy controls. This has not been investigated earlier.

Longitudinal studies are preferred when estimating changes in pain perception between the different phases (57). We have earlier reported preictal heat suballodynia, i.e. a pain threshold decrease within the normal range (see Weissman-Fogel et al. (12) for a discussion of the term), in migraine patients (36). However, the number of migraineurs with both interictal-ictal and interictal-postictal paired measurements was too low to be analyzed in our previous study published in 2008 (36).

The present blinded longitudinal study included a larger number of migraineurs with both interictal-ictal and interictal-postictal paired measurements. We test the hypothesis that pain thresholds decrease and pain scores increase both the day before, during and the day after the ictal phase compared to the interictal phase, indicating that suballodynia and/or hyperalgesia precedes and outlasts the headache during migraine attacks. Secondly, we test the hypothesis

that migraineurs in the interictal phase have lower pain thresholds and increased suprathreshold pain scores than headache-free controls.

Methods

We measured thermal pain thresholds once a week for four weeks in migraineurs (mean ± SD: 6.7 ± 1.9 days between sessions) in the period between June and December 2012. The migraineurs completed a headache diary for four weeks before, during and four weeks after the examinations in order to determine how the examinations were related to the migraine attacks (i.e. interictal, preictal, ictal or postictal). Thermal pain thresholds and scores were measured once in headache-free controls.

Subjects

Fifty migraineurs and 31 headache-free controls were recruited by advertising in the local newspaper, on the local hospital's webpage (St. Olavs Hospital, Trondheim University Hospital; www.stolav.no/seksjon-engelsk) and on the Intranet within our university (NTNU, Norwegian University of Science and Technology; www.ntnu.edu).

Controls could have headache less than once a month. If they had any occasional headache we asked if they had consulted a physician regarding headache, if the headache was experienced as painful and if they used abortive medication for their headache. They were excluded if they confirmed more than one of these three questions. Forty control subjects were screened over

telephone, two did not meet the criteria and seven dropped out. Thus, a total of 31 controls completed one examination each.

Migraineurs were evaluated by neurologists according to the ICHD-II criteria for migraine with or without aura (49). Included subjects had an attack frequency between two and six per month and had no more than ten days with migraine attacks per month. They could use symptomatic, but not prophylactic migraine treatment. Exclusion criteria were coexisting tension-type headache seven days or more per month in migraineurs, neurological or psychiatric diseases, sleep disorders, active infectious diseases, connective tissue diseases, metabolic, endocrine or neuromuscular diseases, other clinically relevant painful conditions including recent injuries, malignancy, previous craniotomy or cervical spine surgery, heart disease, cardiopulmonary or cerebrovascular diseases, pregnancy, medication for acute or chronic pain, antipsychotics, antidepressants, anticonvulsants or other drugs that may influence neuronal, vascular or muscular function, alcohol or drug abuse, ferromagnetic implants and prophylactic allergy treatment.

One migraineur withdrew consent after the first examination and was not included in the analysis. Three migraineurs attended only once, twice and three out of four times respectively. Forty-nine migraineurs completed a total of 190 examinations (Figure 1). Table 1 shows demographic and clinical data.

Investigators were blinded to diagnosis on subjects' first visit and to migraine phase on the subsequent visits. Inclusion, coordination and follow-up of participants were done by co-

workers, and participating subjects were specifically told not to reveal which group they belonged to the investigators. The Regional Committees for Medical and Health Research Ethics approved the protocol and all subjects gave their written informed consent. Migraineurs and controls received an equivalent of $ 125 and $ 30 respectively, to cover expenses.

Procedure

All sessions in one subject were on the same time of day. The method of limits was used to measure thermal pain thresholds (51). Recordings were performed on SOMEDIC SenseLab equipment (Somedic Sales AB, Stockholm). The right hand (thenar eminence overlying the abductor pollicis brevis muscle) and right side of the forehead (frontal region above the eyebrows aligned with the inner canthus) were stimulated with a hand-held rectangular 25 x 50 mm Peltier element thermode (Somedic Sales AB, Stockholm). Target start temperature was 32 °C and the actual start temperature was recorded by the system. The stimulation range was 5-50 °C and the slope was 1 °C/s. Cold pain threshold (CPT) and heat pain threshold (HPT) were measured four times consecutively with 4-6 seconds random inter-stimuli intervals. The order was constant; CPT before HPT and hand before forehead. Participants were instructed to press a button when the stimulus was perceived as painful. An introductory round was carried out at the beginning of each the day, consisting of two measurements of both thresholds on the hand.

Temporal profiles of suprathreshold heat pain scores were obtained during 30 seconds continuous suprathreshold heat pain stimulation on the right forearm and temple. The

individual determined tonic temperature that scored 6 on a verbal numerical rating scale (NRS) ranging from 0 = “no pain” to 10 = “unbearable pain” was set as the test stimuli (52). We used the same equipment and thermode as when testing thresholds, controlled by the software Exposure30 by SOMEDIC. Start temperature was set at 32 °C, slope 1 °C/s. To determine a temperature level for the test stimulus, subjects were first exposed to stimuli of seven seconds duration at 45 °C. They verbally reported pain scores using NRS continuously throughout stimulation. The highest pain score reported determined the temperature for the next test stimulus. We increased the temperature if NRS was less than six and decreased if NRS was more than six. At least three stimuli were applied on both sites with a minimum of one-minute inter-stimulus interval on the same site. The temperature perceived as an NRS score closest to six was chosen for the test stimulus. Two temperatures were determined, one for the temple and one for the forearm. The main suprathreshold heat pain test procedure consisted of one continuous stimulation per site with 30 seconds duration. Verbal NRS scores were reported continuously. They were instructed to update their pain score verbally whenever the experienced pain changed. The last reported NRS score at 0, 10, 20 and 30 seconds was stored for analysis, where 0 seconds represents the time the thermode reached the test stimuli temperature. The same individually determined temperatures were used for the next three examination days.

Data analysis

Thresholds were defined as difference from the measured start temperature (dCPT = start – CPT and dHPT = HPT – start). Outlier detection software was applied, removing single dCPT and dHPT responses with magnitude more than three times or less than one third of the mean of the three associated responses from the same examination day. Examinations were classified by the headache diary as interictal (more than one day before attack onset or one day after the attack ended), preictal (less than one day before attack onset), ictal (migraine headache during examination) and postictal (less than one day after the attack ended). A secondary set of analyses were also performed with a three-day limit. Eleven of the 190 examinations were unclassifiable and excluded from data analysis, mainly because they had attacks both the day before and the day after examination. The distribution of phases is shown in Figure 2.

STATA (StataCorp LP, version 13.1) was used to run separate multilevel models (53) for each response variable (dCPT, dHPT and suprathreshold heat pain scores). Inclusion of fixed effects was determined by the research questions. First three models compared migraineurs' within-subject change by migraine phase and site. In addition, to explore adaptation and sensitization effects, we included pain rating-time to explore possible differences within each time-point of the continuous suprathreshold heat pain stimulation protocol. Secondly, in three models we compared between-group responses from controls and migraineurs in the interictal phase.

The lower limit of the thermal threshold equipment was 5 °C, i.e. dCPT = 27. A substantial number of dCPT-measurements reached this limit. We knew that these dCPT were above 27,

171 but not by how much, and they were thus defined as censored (54). The distribution of
172 censored responses was skewed, e.g. more in controls than interictal migraineurs. One may
173 underestimate a possible difference between the groups if the censored variables are not
174 properly accounted for. Analysis of dCPT was done by modeling both the change of non-
175 censored responses between phases and the probability of reaching the limit, while
176 accounting for dependencies in the data, see the appendix for details.

177 Level one residuals and empirical Bayes estimates of higher-level random effects were plotted
178 on histograms and qq-plots to check the distributions. dHPT was squared to improve normality
179 of residuals. Full model specifications are detailed in the appendix. Individual temperatures
180 used for suprathreshold tonic heat stimulation were compared between groups with
181 independent Student's t-tests. Results were considered significant at a level of $p < 0.05$. Note
182 that predicted values from multilevel modelling, reported in figures and in the text below
183 (presented as coefficients with associated 95% CIs), will not be identical to mean values
184 reported in Table 2 and 3.

185 As additional secondary sub-analyses, we extended the models with selected factors and
186 covariates that might have had an effect on the results. Aura and headache lateralization were
187 tested as factors. Differences in summation of pain thresholds between phases and groups
188 were tested by including a linear covariate of test repeats.

189 To test if there was a linear relationship between pain thresholds and scores and time to the
190 next attack, three additional multilevel models were conducted. They were specified the same

way as the three main models except the dummy-coded variable “phase” was exchanged with the continuous variable “days to next attack”. Interictal recordings were first analyzed, while preictal and interictal recordings were included in a second set of analyses.

With 30 controls and 50 migraine subjects, the power to detect a low medium-sized effect equal to 0.65 SD (55) based on a two-sample t-test was calculated to 80 %. As we estimated to have approximately 20 pairs for intraindividual phase-related comparisons, power (based on paired t-tests) to detect a similar medium-sized effect (0.65 SD) was calculated to 83 %.

Results

Migraineurs by phase

Table 2 shows descriptive means of dCPT, dHPT and pain scores by phase and site. Forehead dCPT decreased by 2.2 [95% CI: 0.5, 4.0] °C (p = 0.014) in the ictal phase compared to the interictal phase (Figure 3). The interictal-ictal forehead dCPT-change was significantly larger than the interictal-ictal change at the hand (p = 0.013). Neither preictal nor postictal dCPT changed compared to interictal dCPT. Post-hoc analysis of contrasts shows that ictal forehead dCPT were significantly decreased compared to both preictal (p = 0.043) and postictal (p = 0.037) dCPT. These findings were interpreted as ictal forehead suballodynia. There were no significant hand or forehead dHPT differences between phases (p > 0.10, Figure 4).

Overall pain scores to the continuous suprathreshold heat pain stimulation at the temple was 0.6 [95% CI: 0.1, 1.2] points higher ictally compared to interictally (p = 0.031). When looking at

the pain scores separately for each time point, lower scores were found preictally for the first time point. Temple pain scores at 0 seconds were 0.8 [0.2, 1.4] and forearm scores 0.7 [0.1, 1.3] points lower in the preictal compared to the interictal phase ($p < 0.029$, Figure 5). Less adaptation was found preictally compared to interictally, as pain scores at both sites decreased from 0 to 20 and 30 seconds in the interictal phase ($p < 0.001$), while preictal pain scores decreased significantly less ($p < 0.020$).

Neither dCPT, dHPT nor pain-score results were significantly altered by controlling for aura or headache laterality. Both dCPT and dHPT showed a significant linear summation of pain during the four stimuli ($p < 0.001$). However, the summation did not differ between phases ($p > 0.079$) and did not alter the original results.

Days to the next attack did not affect dCPT and dHPT neither for the interictal group nor the combined interictal and preictal group ($p > 0.34$). For the interictal subgroup a daily increase in pain score towards the next attack was estimated to 0.08 [0.01, 0.15] ($p = 0.033$) on the temple and 0.09 [0.02, 0.16] ($p = 0.008$) on the forearm. However, when preictal recordings were added the significant association disappeared. Adaptation of pain scores from 0 to 20 and 30 seconds remained significant in both analyses ($p < 0.004$).

For dCPT and dHPT changing the definition of the preictal and postictal phases from a one-day limit to a three-days limit did not change the original results. However, preictal pain scores at 0 seconds and the adaptation from 0 to 20 and 30 seconds were then no longer significantly different between the interictal and preictal phase ($p > 0.79$).

Interictal migraineurs and controls

Table 3 shows descriptive means of dCPT, dHPT and pain scores by group and site. Hand dCPT was decreased by 4.4 [0.7, 8.1] °C ($p < 0.019$) in interictal migraineurs compared to controls. Forehead dCPT was not different between groups ($p = 0.76$). Neither dHPT nor pain scores differed significantly between groups ($p > 0.11$). Pain scores during continuous suprathreshold heat pain stimulation decreased in both groups from 0 to 20 and 30 seconds ($p < 0.001$). Test stimulus temperature means (± SD) were also not significantly different between migraineurs and controls (temple: 46.7 ± 1.9 vs. 46.9 ± 2.1 °C, $p = 0.69$, forearm: 45.9 ± 1.8 vs. 46.5 ± 2.1 °C, $p = 0.22$).

Discussion

We observed trigeminal cold suballodynia and heat hyperalgesia during the ictal phase. Pain thresholds did not change from the interictal to the preictal or postictal phase. This finding indicates that initial cortical processes responsible for the prodromal symptoms is not associated with substantial sensitization of extracranial thermal nociceptors, at least not until the actual headache phase is rather close.

In line with the previously reported ictal thermal allodynia (18), preictal heat and cold suballodynia (36), increased nociceptive activity in the spinal trigeminal nuclei (44) and decreased HPT towards the next attack (10), one would expect that pain thresholds gradually decrease and pain scores increase from the interictal to the preictal and subsequently to the

ictal phase. Schwedt et al. (10) found an association between decreased forehead HPT and closeness to the next attack in accordance with Sand et al. (36). Another small study did not find significant differences in pressure and thermal pain thresholds between interictal, preictal and postictal migraineurs (1) but the latter study did not possess sufficient statistical power to disprove the concept. Pain thresholds did not change from the interictal to the preictal phase in the present study and we could accordingly not confirm our previous result regarding preictal thermal suballodynia (36). However, both dHPT and dCPT means were lower in ictal compared to interictal phase (Table 2), suggesting that an interictal-preictal-ictal gradient can exist. Although pain thresholds were not affected linearly by days to next attack when interictal and preictal patients were combined and analyzed over a 15-day time-range, it is still possible that preictal thermal suballodynia evolves closer to the attack, e.g. within some hours, in many episodic migraine patients.

The present results may also suggest that preictal abnormalities in heat pain processing may be more consistently expressed as subtle suprathreshold pain score differences. Surprisingly, preictal pain scores demonstrated hypoalgesia compared to interictal scores, which was the opposite of what we expected. However, the pain scores at 0 seconds were no longer lowered preictally when changing the definition of the preictal phase from one to three days before the attack. In fact, the subanalysis with the linear effect on days to next attack showed increasing pain scores closer to the attack when the data from the preictal phase were excluded. Thus, migraineurs had increasing hyperalgesia towards the next attack and hyperalgesia during

269 headache, as expected. However, this general pattern was interrupted for a limited time-
270 window preceding headache, interpreted as preictal hypoalgesia. These results suggest that
271 significant central events affect processing of pain on the day before headache.

272 Stankewitz et al. (44) found lower fMRI-activation in response to trigeminal pain in the spinal
273 trigeminal nuclei in interictal and ictal migraine subjects compared to controls, while activation
274 was normal in the preictal group within 72 hours before the next attack. However, pain scores
275 were unaltered between phases (44). A recent study scanned one migraineur daily for 30 days
276 to analyze fMRI-activation by phase, in response to trigeminal pain (27). The migraine patient
277 experienced three attacks during the period and results showed that hypothalamic activity
278 increased towards each migraine attack. Further, functional coupling analyzes showed
279 increased coupling between hypothalamus and the spinal trigeminal nuclei preictally (24h
280 limit), whereas during the ictal phase, coupling to the trigeminal nuclei was significantly
281 decreased (although increased between hypothalamus and the dorsal rostral pons) (27). These
282 results, combined with the preictal hypoalgesia observed in our study, may suggest that fMRI-
283 activation of the trigeminal nuclei reflect increased descending modulation preictally (26).
284 Preictal hypoalgesia was present both in the face and in the arm in the present study,
285 supporting that preictal pain scores are altered by central rather than peripheral mechanisms.

286 The observed temporal profile of pain scores during continuous suprathreshold heat pain
287 stimulation in the present study is at variance with some (52, 56, 57), but not all previous
288 studies (58-62). Migraineurs demonstrated lower initial pain and significantly less adaptation in

289 the preictal compared to the interictal phase. A-delta fibers may be important for the initial
290 rise and fall in pain scores observed the first fifteen seconds of the continuous suprathreshold
291 heat pain stimulation (60, 63, 64). Our observed lower pain scores could have reflected a
292 blunted preictal A-delta nociceptive response, but since a central mechanism is most probable,
293 we interpret this finding as a blunted preictal saliency perception.

294 The decreased hand dCPT in migraineurs between attacks compared to controls may reflect a
295 state of slight chronic sensitization of pain pathways, possibly due to frequent pain
296 experiences (43) as pain thresholds may decrease in relation to increased attack frequency
297 (65-67). Cortical pain modulation seems to be disturbed in migraine (68). Altered sensory
298 modulation in general is also reflected by phono- and photophobia, prodromal symptoms (19,
299 23), and migraine triggers like cognitive stress (69) in susceptible subjects (70). However,
300 enhanced interictal sensitization was of moderate magnitude in our present study, as only
301 hand CPT was affected, indicating that pain thresholds and pain scores may be largely
302 unaltered interictally. In accordance with a previous study (12), pain scores to tonic
303 suprathreshold heat did not differ between interictal migraineurs and controls. Overall,
304 thermal pain sensitivity changes in migraine may be easier to observe in the cold than the heat
305 domain.

306 Studies comparing experimental pain in migraineurs and controls have shown variable results;
307 either hypersensitivity (1-13) or no differences (1, 5, 9, 12, 36, 67, 68, 71-76), but never
308 hyposensitivity. Some subgroups may be more hypersensitive than others; for instance,

309 migraineurs with non-sleep related migraine attacks had lower CPT and HPT than controls (77),
310 while less slow wave sleep was associated with higher pressure pain thresholds (1). Disease
311 severity may also be of importance, as headache history duration may modulate CPT (36),
312 while chronic migraineurs (> 15 days/month) were more sensitive to pain compared to
313 episodic migraineurs in one study (66), but not in another (9). Headache frequency correlated
314 with temporal summation of electrical and mechanical stimulation (12) and pressure
315 thresholds (67), although there are contradictory findings (4). Thermal pain thresholds did not
316 correlate with headache frequency, allodynia symptom severity, anxiety scores or depression
317 scores (10). Migraine is divided into subgroups of subjects with and without aura, but these
318 groups did not differ in the present study and do not seem to differ systematically by pain
319 thresholds in previous studies (5, 36). Thus, since a multitude of factors may influence
320 sensitivity in individual patients, this heterogeneity may explain why results regarding pain
321 thresholds and other sensitivity measures vary between studies.

322 ## Strengths and limitations

323 By prospectively measuring pain thresholds and scores four times within each patient, we
324 obtained a substantial number of subjects measured at different phases. Blinding of the
325 investigators during recording and analysis adds further strength to the study (78). We used
326 robust and flexible multilevel statistical models, enabling us to analyze all the data without
327 prior mean calculations and listwise deletions, optimize the model fit and to properly account
328 for the substantial and uneven censoring of dCPT between groups. An alternative study design,

as asking patients to present for a test session during attack, would increase the number of ictal recordings and thus power for an interictal-ictal comparison, although it would be more difficult to control factors like time of day, blinding of phase and anticipation. But more importantly, we chose random recordings with diary-based classification to be able to investigate the preictal phase.

To obtain reproducible results, we applied a standardized procedure (79); the room was quiet with constant lightning (no windows), pre-written instructions were read to all subjects, the test was always done in the same manner and the same examiner did all the testing. The repeatability of thermal pain thresholds has proven to be satisfactory, although CPT may be a less robust measure due to relatively large standard deviations (80-82).

The comparisons of interictal migraineurs and controls could have been biased by habituation/sensitization effects because we included all the exams of migraineurs in the interictal phase. However, the conclusions did not change by rerunning the analyses with only exams from the first day (results not reported).

We tested the pain thresholds and scores systematically on the right side, regardless of which side the migraineurs most commonly experienced headache. This may be a drawback since allodynia ipsilateral to the headache may occur before contralateral allodynia (83). However, a previous study demonstrated no significant difference between the symptomatic and non-symptomatic side for the interictal-preictal differences (36) and inclusion of headache laterality in the subanalyses did not affect the results. Migraineurs were allowed to take

abortive medications. However, it is unlikely that the medication has effect on other phases than the ictal phase due to short half-life, and the effect is likely to be increased pain thresholds and decreased scores, the opposite of what we found in the ictal phase. Six of the migraineurs reported prodromal allodynia by questionnaire. We did not collect information on self-reported clinical interictal or ictal allodynia, an explanatory variable that could be of importance.

Repetitive painful stimuli evoke pain amplification characterized by increased responses in the dorsal horn and in descending modulation of pain (84). The central mechanisms to pain amplification may be common for both phasic and tonic pain (52). We obtained temporal profiles during 30 seconds of suprathreshold heat stimulation. Future studies should extend the stimulation period in order to analyze pain intensification during the second minute of tonic heat stimulation (57, 62) and further elucidate variations in central pain modulation between phases.

Conclusion

The present longitudinal study is unique by recording experimental pain from patients at four different occasions, aiming to perform intraindividual analysis of the most clinically relevant pain-physiology parameters (reflecting hypo- hyperalgesia/allodynia/ temporal summation) by migraine phase from a sufficiently large sample. We found trigeminal cold suballodynia and heat hyperalgesia during the ictal phase of migraine headache, and heat hypoalgesia and reduced adaptation to tonic suprathreshold heat pain preictally in both trigeminal and

peripheral sites. Our findings suggest that central modulation of pain depends on migraine phase. Although the ictal phase is characterized by increased trigeminal pain sensitivity, different (and subtle) changes were found in the preictal phase; possibly due to increased descending pain modulation affecting mainly suprathreshold pain scores. Our results support the theory that migraine is a cyclic disorder of the central nervous system related to global alterations of brain excitability and homeostasis. Studies with an emphasis on the preictal phase, preferably longitudinally with high temporal resolution and with parallel paraclinical recordings using fMRI, etc., are needed to further elucidate migraine pathogenesis.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

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Article highlights

- This blinded longitudinal study investigated within-subject fluctuations of thermal pain sensitivity by migraine phase.
- We found heat hypoalgesia on the day before headache, as suprathreshold pain scores were decreased.
- We found cold suballodynia and hyperalgesia during headache, as cold pain thresholds were decreased and suprathreshold pain scores were increased.
- Cyclic central changes in pain physiology seem to emerge during the preictal phase, possibly related to headache-initiating mechanisms.

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597

598 Table 1. Demographic and clinical data after exclusions.

	Controls (*n* = 31)	**Migraineurs (*n* = 49)**
Age mean (SD) [range], years	38 (12) [21-59]	40 (10) [19-62]
BMI mean (SD), kg/m^2	25 (3)	26 (3)
Women, *n* (%)	26 (84)	41 (84)
Days since 1st day of last menstrual period mean (SD)	19 (10)	17 (12)
MwoA, MA+MwoA, MA, *n* (%)	NA	27 (55), 18 (37), 4 (8)
Years with headache mean (SD) [range]	NA	21 (9) [1-40]
Migraine days/month mean (SD) [range], 0-4[a]	NA	1.8 (0.6) [1-3]
Migraine intensity mean (SD), 1-4[b]	NA	2.5 (0.6)
Headache duration mean (SD) [range], hours[c]	NA	16 (21) [0.5-72]

[a] Migraine days/month: 0: < 1/month, 1: 1-3/month, 2: 4-7/month, 3: 8-14/month, 4: > 14/month.
[b] Migraine intensity: 1: Mild, 2: Moderate, 3: Severe, 4: Extreme.
[c] Average duration of an attack with or without use of symptomatic medication.
MwoA: migraine without aura. MA+MwoA: some attacks with and some without aura (both diagnoses according to ICHD-III (50)). MA: migraine with aura (in 100 % of attacks). NA: not applicable.

599

600

Table 2. Observed mean (SD) thermal pain thresholds and pain scores by migraine phase and stimulation site.

			Cold pain thresholds*		Heat pain thresholds		Pain scores	
	N	***n***	**Forehead**	**Hand**	**Forehead**	**Hand**	**Temple**	**Forearm**
Interictal	44	105	16.6 (7.5)	20.0 (6.1)	11.8 (3.8)	12.4 (4.3)	4.0 (1.8)	4.1 (1.6)
Preictal	27	37	16.9 (7.9)	20.2 (5.5)	12.0 (3.9)	13.2 (3.9)	3.8 (1.9)	3.9 (1.7)
Ictal	20	22	13.9 (7.0)	19.5 (5.5)	11.5 (4.2)	12.3 (3.7)	4.7 (2.3)	4.4 (1.9)
Postictal	13	15	16.5 (5.7)	21.4 (6.8)	12.5 (4.0)	13.5 (4.2)	4.5 (1.9)	4.6 (1.7)

Thresholds are expressed in mean °C difference from start temperature (32 °C), scores in mean pain during 30 seconds of tonic heat, measured using a numerical rating scale ranging from 0 = "no pain" to 10 = "unbearable pain".

N: number of subjects with at least one recording at the respective phase.

n: total number of measurements by phase**.**

* The dCPT-means are calculated including the measurements that reached the predefined limit at 27 and are thus not directly comparable to the predicted means from the multilevel model, se appendix for further description.

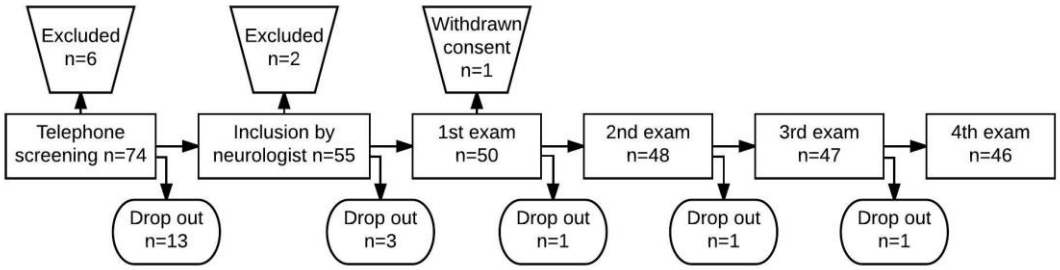
612 Table 3. Mean (SD) thermal pain thresholds and pain scores in interictal migraineurs and
613 controls.

		Cold pain thresholds*		Heat pain thresholds		Pain ratings	
	N	**Forehead**	**Hand**	**Forehead**	**Hand**	**Temple**	**Forearm**
Migraine	44	17.0 (7.3)	20.5 (6.0)	12.3 (3.9)	12.9 (4.5)	3.5 (2.1)	3.2 (2.0)
Control	31	17.5 (7.6)	23.3 (5.1)	12.5 (4.2)	14.1 (4.2)	4.1 (1.9)	3.8 (2.4)

614 Thresholds are expressed in mean °C difference from start temperature (32 °C), scores in mean
615 pain during 30 seconds of tonic heat, measured using a numerical rating scale ranging from 0 =
616 "no pain" to 10 = "unbearable pain".
617 *N:* number of subjects whithin each group.
618 * The dCPT-means are calculated including the measurements that reached the predefined
619 limit at 27 and are thus not directly comparable to the predicted means from the multilevel
620 model, se appendix for further description.
621

622

623 Figure 1

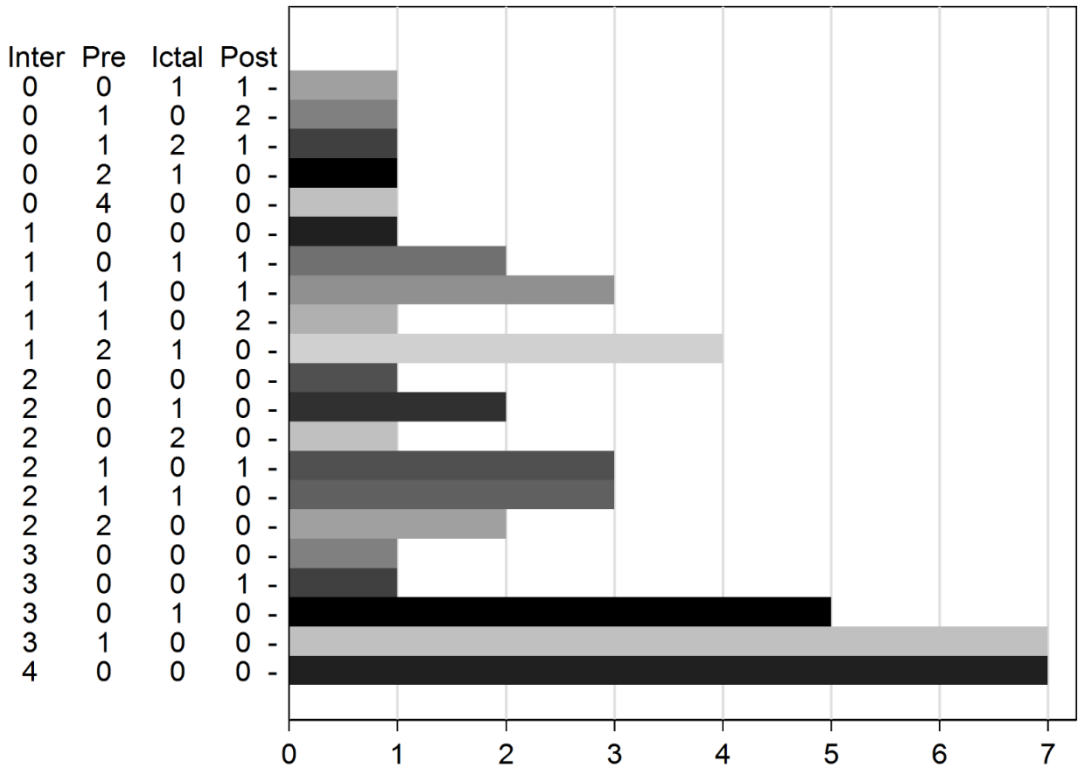


624

625 Flow chart for the migraineurs in the study. The number of subjects who dropped out due to
626 personal reasons are shown at the bottom.

627

628 Figure 2

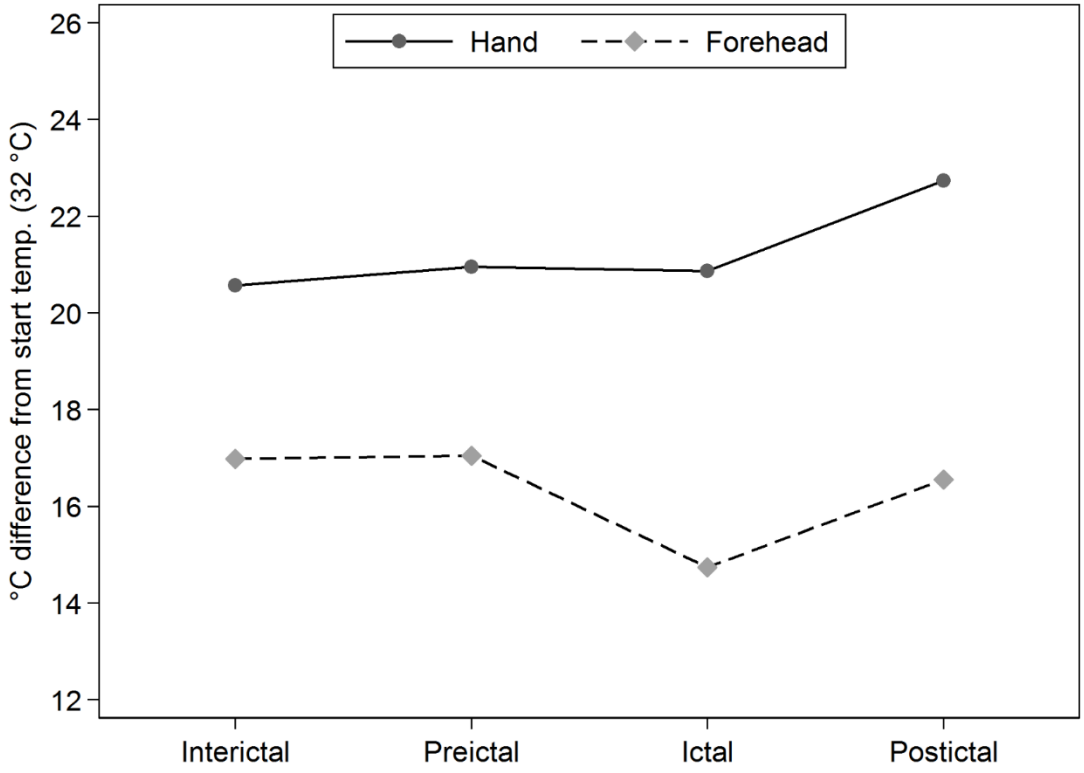


629

630 Bar graph showing the distribution of phase combinations among migraineurs. The labels on
631 the y-axis represents the number of exams in each phase (interictal, preictal, ictal and
632 postictal, respectively). Hence, e.g. 2,1,0,1 means two interictal, one preictal, zero ictal and
633 one postictal recording. The number of subjects with a particular combination of phases are
634 represented by the size of the corresponding bar and labeled on the x-axis. Drop-outs account
635 for 6 missing tests, while 11 tests were excluded as unclassifiable.

636

637 Figure 3

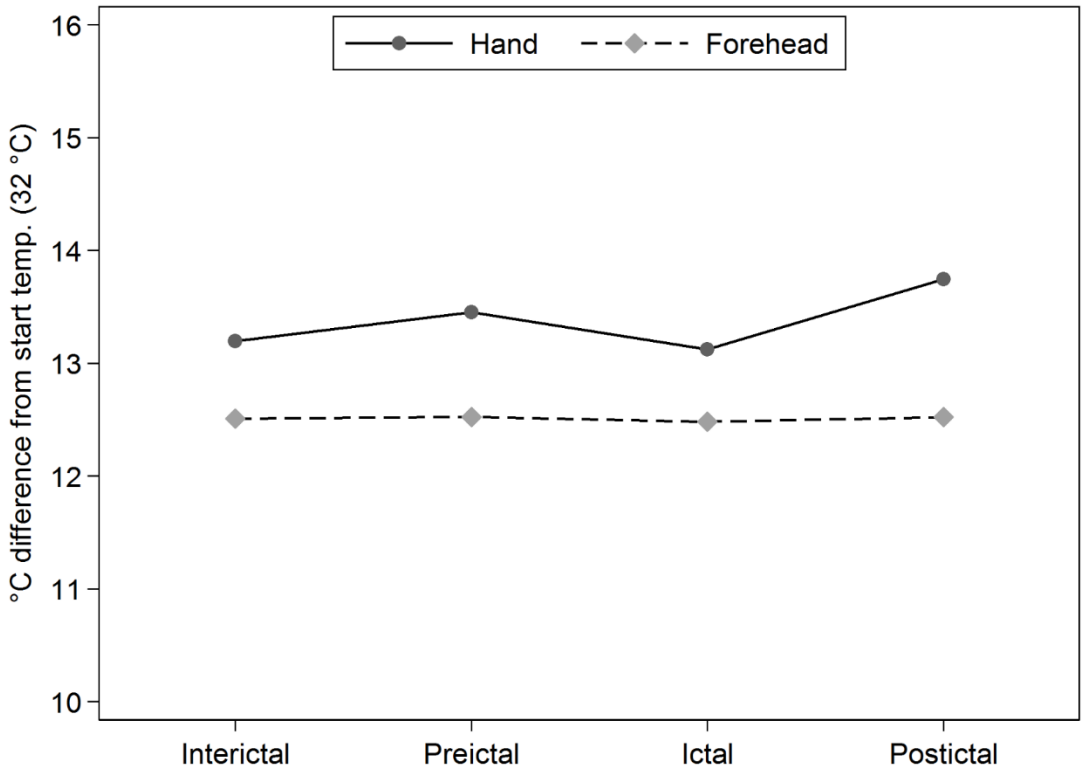


638

639 Cold pain thresholds. Graphical display of estimated margins from the main multilevel model
640 comparing the effects of phase and site on cold pain thresholds. Ictal forehead thresholds
641 were significantly decreased compared to interictal forehead thresholds. The decrease was
642 within the normal range, thus interpreted as trigeminal suballodynia in the ictal phase.

643

644 Figure 4

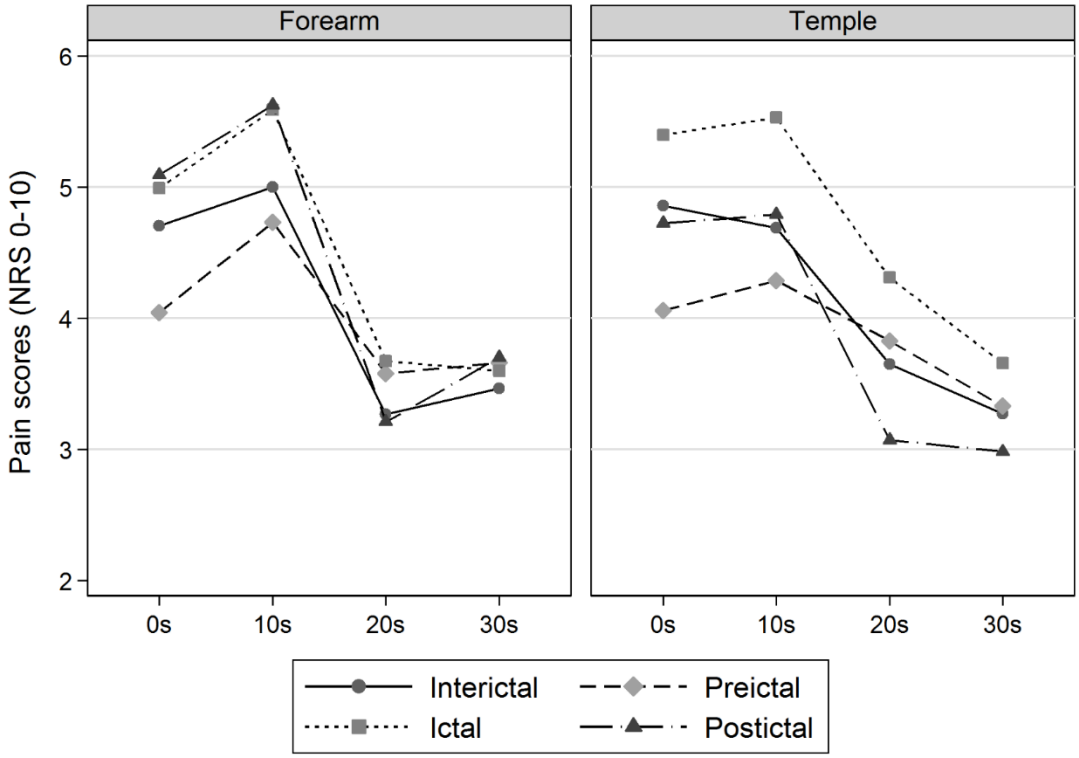


645

646 Heat pain thresholds. Graphical display of estimated margins from the main multilevel model
647 comparing the effects of phase and site on heat pain thresholds. There were no significant
648 differences between phases.

649

650 Figure 5



651

652 Pain scores during continuous suprathreshold heat pain stimulation. Graphical display of
653 estimated margins from the main multilevel model comparing the effects of phase, site and
654 time on pain scores. The x-axis represents the four time-points at which pain scores were
655 recorded during 30 seconds of tonic heat. The overall pain scores at the temple were increased
656 ictally compared to interictally, interpreted as trigeminal hyperalgesia in the ictal phase. At
657 time point 0s preictal pain scores were decreased at both sites compared to interictal pain
658 scores, interpreted as initial preictal hypoalgesia. There were interictal decreases in pain scores
659 from 0s to 20s and 0s to 30s at both sites, interpreted as interictal adaptation of pain scores.
660 The preictal pain scores decreased significantly less, interpreted as preictal lack of adaptation.

661

Appendix

We used multilevel analysis, also known as hierarchical linear models, mixed models, and random coefficient models (1) to analyze the repeated measures data in the present study. This enabled us to use all the available data with greater flexibility and to properly account for within-subject and within-day correlations (2).

As stated in the paper, we used STATA (StataCorp LP, version 13.1) to run separate multilevel models for each response variable (dCPT, dHPT and pain rating). We included fixed effects according to the research hypotheses. The main effects of phase and site and their interaction were included to analyze the within-subject pain thresholds. Phase was dummy-coded with the interictal phase as baseline in order to separately compare preictal, ictal and postictal with interictal responses. In addition to these two fixed effects, the pain rating analysis included time of pain rating (0, 10, 20 and 30 seconds, dummy-coded with 0 seconds as baseline) and the two-way interactions between time and phase, and time and site. The three-way interaction was non-significant and omitted to simplify interpretation of the two-way interactions of main interest. Contrasts were used to further explore significant main effects and interactions post-hoc.

To properly account for correlations in the data, we intended to analyze the data as three-level models. The four repeated measurements of each threshold from the same day are probably more correlated than between days, and measurements within each subject are certainly more correlated than between subjects. Thus, measurements are nested in days nested in

682 subject. The likelihood ratio test was used to justify inclusion of random effects and to specify
683 covariance structures. We used Akaike and Bayesian information criterions to compare non-
684 nested models. Level one residuals and empirical Bayes estimates of higher-level random
685 effects were plotted on histograms and qq-plots to check the distributions. dHPT was squared
686 to improve normality of residuals.

687 The analyses of interictal migraineurs and controls were specified with the same fixed effects
688 as the within-subject analyses, but the within-subject factor phase was substituted with the
689 between-subject factor group. These models were defined as two-level models with
690 measurements nested in subjects.

691 More than 15 % of the CPT-responses reached the hardware limit at 5 °C, i.e. dCPT = 27. These
692 responses were defined as censored since we knew that they were above 27, but not by how
693 much (3). Censoring may lead to biased parameter estimations if not appropriately accounted
694 for (4). The Tobit model is an acknowledged and frequently used model for censored data (3,
695 5), and can be extended to longitudinal and repeated measures data (4, 6, 7). We modeled
696 both dCPT multilevel analyses within the generalized structural equation model framework (8,
697 9) with right-censoring specified at 27. The model was fitted with a sandwich estimator
698 correction method to produce robust standard errors (10, 11). The dHPT and pain rating-
699 models were not substantially biased by censoring and were thus fitted as regular multilevel
700 models with restricted maximum likelihood estimation.

701 The effect of appropriately accounting for censoring is clearly visible when comparing the
702 difference in the descriptive means (2.8 °C, table 3) and estimated coefficient (4.4 °C) between
703 migraineurs and controls' hand dCPT in the present study. Forty-three percent of hand dCPT-
704 measurements in controls reached the limit and were thus censored, whereas only 23 % of
705 migraineurs' hand dCPT-measurements were censored. The descriptive means were calculated
706 by assigning the value 27 to censored cases. The discrepancy in proportion of censored values
707 between groups will thus lead to a greater underestimation of the dCPT in controls compared
708 to migraineurs, resulting in a smaller mean difference. The Tobit model combines the non-
709 censored cases and the probability of being censored to compute less biased coefficients (3),
710 which in our case resulted in a substantial increase in the group difference.

711 The final dCPT-model was defined as a three-level model with measurements nested in days
712 nested in subjects. A random slope for site with an unstructured covariance structure was
713 added at the second level. The within-subject day-to-day variation of dHPT was not significant
714 different from zero. Thus, the dHPT-model was simplified and defined as a two-level model
715 with measurements nested in subjects. A random slope for site with an unstructured
716 covariance structure was added. The final pain rating-model included Site as random
717 coefficient at the third level with an independent covariance structure and an unstructured
718 residual covariance structure by time of pain rating. The estimated fixed factors are presented
719 in Appendix Table 1 and Appendix Table 2 below.

720 The between-group models were defined as two-level models with measurements nested in
721 subjects. The final dCPT and dHPT-models included site as random coefficient at the second
722 level with an unstructured covariance structure. dHPT-residuals were modeled by site with an
723 autoregressive residual covariance structure by measurement number. The final pain rating-
724 model included site as random coefficient with an independent covariance structure and an
725 unstructured residual covariance structure by time of pain rating.

726

727

728 **Appendix Table 1.** Estimated pain threshold coefficients (standard error).

	Cold pain thresholds	Heat pain thresholds
Phase		
Preictal	0.061	0.352
	(1.00)	(9.71)
Ictal	-2.248*	-0.710
	(0.91)	(11.44)
Postictal	-0.430	0.309
	(0.77)	(14.12)
Site		
Hand	3.584***	17.622*
	(0.82)	(8.03)
Interactions		
Preictal × Hand	0.325	6.443
	(1.28)	(13.07)
Ictal × Hand	2.545*	-1.169
	(1.02)	(15.62)
Postictal × Hand	2.590	14.528
	(1.86)	(18.85)
Constant	17.0	156.5
	(1.20)	(12.27)

729 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
730 Phase and site were dummy-coded with Interictal and Forehead as baseline, respectively.
731 Thus, the constant represents interictal forehead pain thresholds. Pain thresholds are
732 presented as difference from start temperature (32°C). Heat pain thresholds were squared
733 before estimation.
734
735

736 **Appendix Table 2.** Estimated pain score coefficients (standard error).

	Coefficient	Standard error
Phase		
Preictal	-0.798**	0.31
Ictal	0.540	0.36
Postictal	-0.135	0.44
Site		
Forearm	-0.153	0.22
Time		
10s	-0.169	0.20
20s	-1.207***	0.24
30s	-1.587***	0.24
Interactions		
Phase × Site		
Preictal × Forearm	0.135	0.20
Ictal × Forearm	-0.252	0.23
Postictal × Forearm	0.522	0.28
Phase × Time		
Preictal × 10s	0.395	0.31
Preictal × 20s	0.976**	0.37
Preictal × 30s	0.858*	0.37
Ictal × 10s	0.300	0.38
Ictal × 20s	0.118	0.45
Ictal × 30s	-0.155	0.45
Postictal × 10s	0.237	0.44
Postictal × 20s	-0.444	0.53
Postictal × 30s	-0.153	0.53
Site × Time		
Forearm × 10s	0.464	0.24
Forearm × 20s	-0.231	0.29
Forearm × 30s	0.347	0.29
Constant	4.9	0.30

737 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
738 Phase, site and time were dummy-coded with Interictal, temple and 0s as baseline,
739 respectively. Thus, the constant represents interictal temple pain scores at 0 seconds.
740

741

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