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Experimental pain and migraine

Investigating the effect of non-invasive cortical modulation of pain, and phasic alterations of pain in migraineurs

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Thesis for the degree of Philosophiae Doctor

Trondheim, May 2018

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Smerteresponser hos personer med migrene

Migrene er den tredje mest vanlige og den sjuende mest invalidiserende sykdommen i verden. Likevel er ikke årsaken til migrene kjent fullt ut og diagnosen stilles kun ut i fra subjektive symptomer. Vi har i to studier undersøkt smerteresponser hos personer med migrene for å forstå mer om sykdomsprosessen. Resultatene tyder på en endring i hvordan hjernen bearbeider smerte hos personer med migrene. Dagen før hodepinen startet fant vi en paradoksal endring i form av lavere smerterespons. Disse funnene, sammenholdt med tidligere funn, tyder på at flere sentrale sentre i hjernen endrer aktivitet *før* selve hodepinen starter. Dette er sentre som bearbeider smerte og andre sanseinntrykk, samt ivaretar kroppens homeostase.

I den første studien sammenlignet vi 26 personer med migrene og 31 friske kontroller. Vi undersøkte om smerteresponsene for kulde- og varmestimulering endret seg forskjellig etter repetert magnetisk stimulering av et «smerteområde» i hjernen (sekundær somatosensorisk korteks). Smertetersklene hos de med migrene ble ikke vesentlig påvirket, til forskjell fra kontrollene som etter magnetisk stimulering tålte større temperaturendring før de kjente smerte.

I den andre studien undersøkte vi smerteresponser hos 49 personer med migrene gjentatte ganger for å se hva som skjer med responser før, under og etter migreneanfall. Vi målte kulde- og varmesmerteterskler, gradering av smerte ved 30-sekunders varmestimulering og elektrisk respons i hjernen etter smertefull laserstimulering på hånda. Personer med migrene opplevde stimuleringen som *mindre* smertefull døgnet rett før anfall. Under anfall var opplevelsen motsatt og stimuleringen i panna var mer smertefull, noe som passer godt med tidligere funn.

Navn kandidat:	Martin Uglem
Institutt:	Institutt for nevromedisin og bevegelsesvitenskap
Veiledere:	Trond Sand og Petter Moe Omland
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Contents

Preface and acknowledgments

In this thesis, we summarize and discuss some of the findings from two studies presented in three papers [1-3]. These studies are part of the Migraine Physiology project in Trondheim (MIGFYS). The basic ideas were; 1) to study cortical excitability and pain physiology, particularly if we could detect changes even in the preictal phase before headache was present, 2) to study sleep dysfunction and its relation to pain physiology, and 3) to investigate if noninvasive magnetic brain stimulation could alter cortical excitability and pain physiology. The project was planned from 2003 and we started data collection for MIGFYS1 in 2005. Some important results have been summarized in early papers about pain physiology and evoked potential changes through the migraine cycle [4-6] and in three doctoral theses about EEG in migraine [7], sleep in migraine and tension-type headache [8], and cortical visual excitability in migraine [9].

We conducted the two studies discussed in the present work in 2010 and 2012. The first study focused on the cortical excitability in migraineurs and the responsiveness to manipulation with non-invasive brain stimulation. In addition to Paper I [1], we have published three other papers on the data collected in 2010 [10-12]. The second study's focus was cyclical alterations of experimental pain and evoked potentials in migraineurs. In addition to Paper II and III [2, 3], one study has been published presenting measures of visual evoked potentials [13].

Many people have contributed to this thesis. First, I want to thank my supervisor **Trond Sand** for his excellent guidance. He has always taken the time to provide thorough answers and proper guidance, despite a busy schedule. It is reassuring to have a supervisor with such in-depth knowledge, experience, and interest in the field of headache, neurophysiology, and statistics. Second, I want to thank **Petter Moe Omland**, initially a fellow student two years ahead of me, who has become a great co-supervisor. I

am very thankful for the opportunity to cooperate with him, and for all the help and thorough feedback he has given me.

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I am sincerely grateful to the **study participants** who donated a significant amount of their time to these studies.

Finally, I thank my wife and best friend, **Jorun**, who have supported me and stuck with me, with daily encouragement and compliments, and for being a wonderful mom for our lovely daughter **Ada Olive**.

Trondheim, January 2018

Martin Uglem

List of papers

Paper I:

Non-invasive cortical modulation of experimental pain in migraine. (2016)

Uglem M, Omland PM, Engstrom M, Gravdahl GB, Linde M, Hagen K, Sand T

Clin Neurophysiol 127:2362-2369.

Paper II:

Does pain sensitivity change by migraine phase? A blinded longitudinal study. (2017)

Uglem M, Omland PM, Nilsen KB, Tronvik E, Stovner LJ, Hagen K, Linde M, Sand T

Cephalalgia 37:1337-1349.

Paper III:

Habituation of laser-evoked potentials by migraine phase: a blinded longitudinal study. (2017)

Uglem M, Omland PM, Stjern M, Gravdahl GB, Sand T

J Headache Pain 18:100.

Summary

Background:
Both clinical symptoms, imaging findings, and neurophysiological measures suggest varying cyclic alterations in CNS-function between, before, during and after the migraine headache. Altered pain perception (preictal and ictal allodynia) seems to be a common non-obligatory clinical symptom in migraine. However, the pathophysiological mechanisms controlling this periodicity are still mostly unknown.

Quantitative sensory testing (QST), including pain thresholds and pain scores during tonic stimulation, and laser-evoked potentials (LEP) are useful measures of pain perception and processing. We explored how these measures change before, during and after migraine headache in a blinded longitudinal study.

Repetitive transcranial magnetic stimulation (rTMS) may alter the activity in stimulated and connected cortical areas. This method can help us understand the contribution of the stimulated cortical regions in migraine pathophysiology. We measured the effect of rTMS to the secondary somatosensory cortex (S2), a cortical area important for pain processing.

The main aim of the studies was to investigate changes in experimental pain measures in migraineurs, both between homeostatic states, here defined by migraine phase, and by external modulation of cortical excitability with rTMS.

Methods:
In the first study, we explored the effect of high-frequency navigated rTMS and sham stimulation to S2 on thermal detection and pain thresholds, and pain scores; comparing 26 migraineurs in the interictal phase with 31 controls.

In the second study, we measured both QST-measures and LEP four times in 49 migraineurs. The four sessions were categorized by migraine diaries and measures obtained between attacks were compared with recordings from the periods before, during and after an attack. Also, we compared interictal

recordings with 30 controls. In both studies, the investigators were blinded during recording and analysis of the data.

Main results:

- RTMS to S2 increased cold and heat pain thresholds in controls compared to migraineurs in the interictal phase.
- Pain scores by prolonged heat stimulation of both forehead and hand decreased in the preictal phase.
- Trigeminal pain scores increased, and cold pain thresholds decreased (higher absolute temperature), during headache.
- There was a subtle lack of habituation of the N1 LEP-amplitude in the ictal phase.

Conclusions:

We found slightly altered central processing of pain in migraineurs. Effects were observed both as altered pain-modulation after cortical stimulation and by changes through the cyclic migraine-phase process. Interictal slight hyperalgesia, followed by short-lasting preictal hypoalgesia and ictal (and partly postictal) hyperalgesia was generally observed. The increased trigeminal pain sensitivity during headache complies with clinical symptoms and previous neurophysiological findings. However, since interictal and preictal alterations were observed for pain elicited from both hand and face, a general alteration in pain processing like central sensitization (as opposed to a regional trigeminal affection), is supported.

The lack of an analgesic effect by navigated rTMS may reflect non-optimal target, increased thalamocortical activation or decreased intracortical inhibition, and/or reduced activity of the descending pain modulation system in migraineurs.

The preictal hypoalgesia may be caused by transient thalamocortical hypoactivity, cortical hypoexcitability or excessive intracortical inhibition, or by increased endogenous analgesia. The well-known autonomic symptoms before and during migraine, combined with the presently observed hypoalgesia, suggest a possible increase in hypothalamic cyclic modulation of both autonomic control and pain processing.

Although there were few significant results, with moderate effect sizes, our findings highlight the preictal phase as a promising focus in further studies.

Abbreviations

ACC	Anterior cingulate cortex
ANOVA	Analysis of variance
CDT	Cool detection thresholds
CPT	Cold pain thresholds
CSD	Cortical spreading depression
DLPFC	Dorsolateral prefrontal cortex
DPINS	Dorsal posterior insula
EEG	Electroencephalography
fMRI	Functional magnetic resonance imaging
HPC	Heat, pinch, and cold cells
HPT	Heat pain thresholds
IASP	The International Association for the Study of Pain
LEP	Laser-evoked potentials
M1	Primary motor cortex
NRS	Numerical rating scale
PAG	Periaqueductal gray
QST	Quantitative sensory testing
rTMS	Repetitive transcranial magnetic stimulation
RVM	Rostral ventromedial medulla
S1	Primary somatosensory cortex
S2	Secondary somatosensory cortex
tDCS	Transcranial direct current stimulation
TMS	Transcranial magnetic stimulation
VAS	Visual analog scale
WDT	Warm detection thresholds

Introduction

More than a thousand articles about migraine have been published each year the last decade, but the knowledge of migraine pathophysiology is still incomplete. Migraine is a cyclical disease, and the phases probably represent different parts of the pathophysiological processes [14]. Migraineurs experience a spectrum of symptoms, symptoms that frequently precede the headache by hours to days. Understanding the physiological processes in the period preceding the attack may potentially give valuable knowledge necessary to understand migraine pathophysiology. This thesis explores the migraine cycle with a longitudinal approach. The work focuses on changes in cortical sensitivity and central pain modulation during the migraine cycle with an emphasis on the preictal phase.

Migraine

Migraine is a widespread disease estimated to affect about a billion people worldwide [15]. It is ranked as the seventh highest cause of years lived with disability overall, and third in the age group 15-49 years, in the Global Burden of Disease Survey 2015 [15, 16]. The high disability score reflects the high prevalence of migraine, but also that sufferers are treated insufficiently [17]. However, the disability score is likely underestimated because it does not account for the interictal burden that may include anxiety, avoidance and other symptoms [18]. The cost of migraine in the society are high compared to other neurologic disorders [19], mainly due to indirect costs [20]. The often severe headache and the associated symptoms are the primary cause of the reduced capacity, but there is also evidence of reversible cognitive dysfunction during attacks [21] that seems to be normal between attacks [22].

The first known accurate description of migraine was done by Aretaeus of Cappadocia (AD 30-90), who described a "one-sided headache with blackness before the eyes, nausea, vomiting, photophobia and osmophobia" that he named *heterocrania* [23], although he also mentioned "death" as a possible outcome [24]. Galen of Pergamon (AD 131-201) coined the Greek term hēmikrania, which means "half skull." The term was translated into Latin by the Romans (hemicranium), and then to migraine by the French in the 14th century [25]. The history of migraine has numerous descriptions of clinical symptoms, both organic and functional, and theories of its pathophysiology [26], as well as various creative suggestions for treatments [27, 28].

Diagnostic criteria for migraine

Today, the diagnosis of migraine is based on patient history and clinical neurological examination. Additional investigations are only recommended if secondary forms of headache are suspected [29]. A typical migraine headache is characterized by unilateral location, pulsating quality and a moderate or severe pain intensity that is aggravated by or prevents physical activity. Also, according to The International Classification of Headache Disorders 3rd edition (Beta version), the duration of a migraine attack should be between 4 and 72 hours without treatment and accompanied by nausea/vomiting or photophobia and phonophobia [30]. An essential requirement is that the diagnosis should not be better accounted for by another ICHD-3 diagnosis.

The criteria subdivide migraine into several groups, the two most prevalent being migraine with and without aura. Most migraineurs with aura have visual aura; a visual disturbance often expressed as a zigzag figure. Aura can also involve other senses, and cause speech or motor deficits. Motor weakness is a special case that is classified as hemiplegic migraine [30]. Diagnostic criteria for migraine with and without aura are shown in Table 1 and 2.

Table 1 *Diagnostic criteria for migraine without aura [30].*

A. At least five attacks fulfilling criteria B–D
B. Headache attacks lasting 4-72 hours (untreated or unsuccessfully treated)
C. Headache has at least two of the following four characteristics:
    1. unilateral location
    2. pulsating quality
    3. moderate or severe pain intensity
    4. aggravation by or causing avoidance of routine physical activity (e.g., walking or climbing stairs)
D. During headache at least one of the following:
    1. nausea and/or vomiting
    2. photophobia and phonophobia
E. Not better accounted for by another ICHD-3 diagnosis.

Table 2 *Diagnostic criteria for migraine with aura [30].*

A. At least two attacks fulfilling criteria B and C
B. One or more of the following fully reversible aura symptoms:
    1. visual
    2. sensory
    3. speech and/or language
    4. motor
    5. brainstem
    6. retinal
C. At least two of the following four characteristics:
    1. at least one aura symptom spreads gradually over ≥5 min, and/or two or more symptoms occur in succession
    2. each individual aura symptom lasts 5-60 min
    3. at least one aura symptom is unilateral
    4. the aura is accompanied, or followed within 60 min, by headache
D. Not better accounted for by another ICHD-3 diagnosis, and transient ischemic attack has been excluded.

Migraine symptoms

Migraineurs may experience symptoms hours to days before the headache attack as well as symptoms that outlast the headache [30]. Some migraineurs can even predict migraine headaches based on preceding non-headache symptoms. Houtveen et al. [31] examined 87 migraineurs that completed a headache diary four times a day for three to six weeks. They identified eight clustered features, including increased sensory sensitivity, pain/stiffness, and fatigue that predominantly were present the last 12 hours before a headache [31]. Giffin et al. [32] made similar findings in a study where migraineurs entered symptoms in an electronic diary. They reported that the most common symptoms during the preictal phase were tiredness, concentration difficulties, and neck stiffness. Interestingly, Giffin et al. [32] found that although migraineurs could report preictal symptoms several days before an attack, the predictive value of these symptoms was rather low until the last 12-24 hours before an attack. Other known preictal symptoms are hyperactivity, hypoactivity, depression, cravings for particular foods and repetitive yawning [30]. Some studies have shown a beneficial effect by initiating treatment during the preictal phase in selected migraineurs [33]. Preictal symptoms are also common in children and adolescents [34]. Clearly, a migraine starts and ends long before and after the headache phase.

Many factors are believed to trigger a migraine attack. A recent literature review determined that stress was the most common trigger factor, followed by auditory stimuli, fatigue, fasting, hormonal changes (in women), sleep, weather, visual stimuli, olfactory stimuli and alcohol [35]. It should be noted that the influence of these triggers on generating migraine attacks are debated, and strict avoidance of triggers is not necessarily the best advice [36-38]. In fact, preictal symptoms and triggers are overlapping, and it is not apparent what comes first.

Pain physiology

The experience of pain involves multiple brain regions and is highly dependent on cognitive and emotional state [39]. A noxious stimulus is transduced into electrical signals by nociceptors and transmitted along axons to the dorsal horn of the spinal cord. Higher centers, collaterals and a variety of other mechanisms modulate the pain signal in the spinal cord. Second-order neurons in the contralateral tracts transmit the signal mainly to the thalamus, which projects to multiple areas of the brain to form a conscious perception of pain [40]. Although the anatomy is quite well defined, the functional interplay between cortical and subcortical sites is puzzling, and the subjective suffering is largely dependent on what the pain means to the individual [41].

Definition of pain

Pain is defined by the International Association for the Study of Pain (IASP) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" [42]. The subjective experience determines the degree of pain, not the stimulus. A noxious stimulus – "a stimulus that is damaging or threatens to damage" – leads to nociception – "the neural process of encoding noxious stimuli" [42]. Given these definitions, it is possible to experience pain without nociception and to measure nociception without the subjective experience of pain. For instance, subjects experiencing massive trauma may feel no initial pain despite intense noxious stimuli and subjects with functional pain syndromes may experience pain without any measurable nociception [43].

Nociceptors and the spinothalamic pathway

Nociceptors are receptors of the peripheral somatosensory nervous system that respond to damaging stimulus or stimulus that threatens to cause harm [42]. A family of cation channels, the transient receptor potential channels, can detect changes in temperatures corresponding to the physiological range of temperatures that humans can discriminate [44]. These receptors can respond to painful thermal, mechanical, and chemical stimuli and may be involved in the pathophysiology of several painful disorders including migraine [45]. Nociceptors can be grouped according to their differential expression of transient receptor potential channels, e.g., channels that confer sensitivity to heat and capsaicin (vanilloid receptor 1; TRPV1), cold and menthol (melastatin receptor 8; TRPM8) and many chemical irritants (ankyrin 1; TRPA1) [46]. Aδ or C-fibers transmit pain and temperature. Aδ-axons are thinly myelinated nerves with a conduction velocity of 5 to 30 m/s, while C-axons are unmyelinated and conduct at speeds less than 1 m/s. Sudden tissue damage is recognized first by a sharp pain transmitted by Aδ fibers, followed by a slow, dull pain transmitted by C-fibers [47, 48].

The fibers responsible for transmission of noxious, thermal and visceral information enter the spinal cord laterally and terminate in the most dorsal portion of the spinal gray matter [49]. Input from C nociceptive neurons is concentrated in laminae I and II, Aδ nociceptive neurons in laminae I and V and Aβ mechanoreceptors in laminae III-V [50]. Second-order projection neurons within laminae I and V constitute the major output from the dorsal horn to the brain, forming mainly the spinothalamic tract [51]. Spinal lamina I neurons mediates not only pain and temperature, but also itch, sensual touch, and muscle and visceral sensations. Pain and temperature sensation may be a part of the "interoceptive

pathway," an afferent neural system that conveys information of the physiological condition of the body primarily needed to maintain homeostasis [52].

Craig et al. [53] differentiated between three main categories of lamina I spinothalamic neurons; cool-sensitive cells, nociceptive-specific cells, and heat, pinch and cold (HPC) cells. The nociceptive-specific cells transmit the sharp, pricking pain, and HPC cells the dull burning pain. The cool-sensitive cells are sensitive to cooling below normal skin temperature, and the response increases linearly with decreasing temperature. However, the response reaches a plateau at 15 °C. HPC cells, on the other hand, responds to cooling below 24 °C and increase the response at noxious cold temperatures. Thus, HPC neurons are likely responsible for the grading of noxious cold, which explains why many describe noxious cold as burning. The cold pain threshold is usually below 15 °C. Although HPC neurons respond from 24 °C, simultaneous activation of cool-sensitive cells probably inhibits a pain sensation at the thalamocortical level. The effect is illustrated by the thermal grill illusion, as reported by Thunberg in 1896 [54]. He made a device that simultaneously stimulated at 20 and 40 °C and yielded a feeling of burning pain, despite both being innocuous temperatures when applied separately. The explanation is thought to be an inhibition of cool-sensitive cells by the warm stimulus—which reduces inhibition of HPC-cells—and a relatively increased HPC activity that yields a burning feeling [53].

Microneurography-studies have identified nociceptors responsive to both heat and mechanical stimuli, only heat stimuli, only mechanical stimuli, and units normally insensitive to both heat and mechanical stimuli [55-58]. "Silent" afferents are electrically excitable but insensitive to mechanical or heat stimulation. However, the silent afferents may become heat or mechanosensitive by inflammation of the receptive field [59, 60]. Studies have demonstrated both cutaneous, visceral, articular, and dural silent nociceptors [55, 58, 61-63]. This subgroup of nociceptors may be important in development and maintenance of hyperalgesia and allodynia after tissue injury or inflammation [64], and they have been found to be spontaneously active in chronic pain [65-67].

Cortical and subcortical pain areas

Functional magnetic resonance imaging (fMRI), positron emission tomography, and electro- and magnetoencephalography studies have identified several cortical regions that respond to painful stimuli. Most studies report activation of the primary (S1) and secondary somatosensory cortex (S2), insula, and anterior cingulate cortex (ACC) [68]. For instance, painful heat stimuli (delivered using a laser) evoked peak fMRI responses in the contralateral anterior and posterior insula, and S2 [69]. S2 is located "along

the superior bank of the Sylvian fissure, lateral and posterior to the face representation in SI and anterior or medial to the primary auditory areas, the medial wall of the temporal lobe, frontal parts of the insula and the parietal operculum" [70]. Some studies have also reported activity in the prefrontal cortex, primary motor cortex (M1), supplemental motor area, and subcortically in the basal ganglia, thalamus, and brainstem [71].

Recent evidence argues that the dorsal posterior insula (DPINS) cortex is fundamental to feelings of pain [72-75], although the pain specificity of this area is debated [76-79]. Pain and temperature, as part of the interoceptive pathway, are transmitted from spinal lamina I to the posterior and basal ventral medial nucleus in the thalamus [80] and terminates at DPINS [72]. Craig [81] thoroughly discusses the structure and function of the insular and cingulate cortices in his recent book. He argues that the insula is functionally divided in three, the primary nociceptive cortex in the DPINS, the integrative region in the mid-insula and the cognitive/emotional region in the anterior insula [82]. Craig further argues that S1 and S2 (at least the lateral part of S2) mainly receives exteroceptive (mechanoreceptive and proprioceptive) signals. Involvement of S1 in nociception is debated [83], and S1-responses to vibration and pain stimuli differs [84]. Intracranial EEG responses to noxious laser stimulation showed activation in the DPINS, operculum (S2), mid-cingulate and amygdala first, followed by activation of anterior insula, and prefrontal and posterior parietal cortices [85]. A follow-up study with extracranial EEG source modeling showed excellent morphological match with the intracranial recording and confirmed the temporal and spatial development of cortical responses to pain [86]. Intracortical recordings of laser responses have also demonstrated different intensity in S2 and DPINS in response to sensory and nociceptive stimuli [87]. Lesions in DPINS and the medial part of S2 disrupt pain and thermal sensation, without affecting proprioception and mechanosensation [88]. Although the interplay between the cortical regions transforming nociception to subjective pain is not clear, an increasing body of evidence shows that DPINS and (medial) S2 play a central role.

The anterior insula and cingulate cortices contribute to attention and awareness of bodily processes [89], including pain [81, 90], and are probably involved in many neurological and psychiatric diseases. For instance, a meta-analysis of 193 studies comprising more than 15 000 individuals found gray matter loss in ACC and insula across several psychiatric diagnoses [91]. Furthermore, a recent review highlighted the involvement of the insular cortex in modulation and chronification of pain [92]. While the DPINS and S2 are thought to be mainly involved in the sensory-discriminative pain coding, ACC and anterior insula are assumed involved in the affective/emotional and cognitive dimensions of pain.

Endogenous modulation of pain

Descending tracts from supraspinal sites modulate the nociceptive inputs in the dorsal horn of the spinal cord. The periaqueductal gray-rostral ventromedial medulla (PAG-RVM) system is the best studied. In 1969, Reynolds demonstrated complete analgesia in rats by electrical stimulation of this area, while the motor function was retained [93]. PAG share interconnections with the hypothalamus and limbic forebrain structures, and have a vital role in coordinating survival strategies. Inhibitory signals from PAG mainly suppress input from C-fiber activation in the dorsal horn, preserving rapidly conducted sensory-discriminative information carried by Aδ nociceptive neurons. PAG projects to the RVM, which projects diffusely to dorsal horn laminae. The RVM constitutes both ON- and OFF-cells, thus it can have both facilitatory and inhibitory effects on the dorsal horn. Higher centers in the central nervous system modulate the PAG-RVM system, e.g., the amygdala activates OFF-cells in response to intense fear or opioid action, contributing to analgesic effects. Stimulation of the ventrolateral orbital cortex, however, activates ON-cells producing hyperalgesia. Other sites known to contribute to the descending control are the dorsal reticular nucleus and ventrolateral medulla [94-99]. Individual differences in pain perception may be a consequence of differences in descending modulation of spinal nociceptive processes from brainstem regions, as shown by fMRI of the brainstem and spinal cord [100].

Pain taxonomy

IASP has made a list of pain terminology in clinical practice, the IASP Taxonomy [42]. We comply with the IASP Taxonomy definitions as presented below. For clarity, when we discuss changes in thermal detection and pain thresholds, we define an increase in threshold as greater distance from the start temperature. Thus, an increased heat pain threshold (HPT) means a change to a higher absolute temperature while an increased cold pain threshold (CPT) means a change to lower absolute temperature. This is convenient as both HPT and CPT decreases will then be interpreted as suballodynia, and increases as hypoalgesia (see below for definitions). In this thesis, we follow this rule when we discuss results from other studies as well as our results.

Sensitization, hyperalgesia, and allodynia

Sensitization is defined as “increased responsiveness of nociceptive neurons to their normal input, and recruitment of a response to normally subthreshold inputs” [42]. The term sensitization applies when we know both input and output of the neural system under study. Thus, clinically, sensitization may only be inferred indirectly from phenomena such as allodynia and hyperalgesia [101]. Allodynia is “pain due to a

stimulus that does not normally provoke pain" [42], a change in quality. Hyperalgesia is "increased pain from a stimulus that normally provokes pain" [42], a shift in the intensity of the response. Hence, an increased response to a painful stimulus is coined hyperalgesia while a pain threshold below the normal range should be termed allodynia. However, we will use the term *suballodynia*, as discussed by Weissman-Fogel et al. [102], to distinguish reduced pain thresholds within the normal range from the standard clinical use of allodynia, that is, thresholds below the normal range.

Classification of pain

IASP has defined three types of pain [42]: 1) *Nociceptive pain*, i.e., "Pain that arises from actual or threatened damage to non-neural tissue and is due to the activation of nociceptors." 2) *Neuropathic pain*, i.e., "Pain caused by a lesion or disease of the somatosensory nervous system." 3) *Nociplastic pain*, i.e., "Pain that arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors or evidence for disease or lesion of the somatosensory system causing the pain." Diabetic neuropathy, postherpetic neuralgia, spinal cord injury, and phantom limb pain are examples of diseases that may cause neuropathic pain. Nociplastic pain is a new descriptor—somewhat debated [103, 104]—meant to comprise findings of altered nociceptive function, without activation of nociceptors or neuropathy, such as fibromyalgia, complex regional pain syndrome, and "functional" pain disorders [105].

Responses to repetitive and tonic pain stimulation

Temporal summation is a measure of sensitization within the central nervous system, and a psychophysical correlate of wind-up, a progressive increased neuronal activity in the dorsal horn by repetitive activation of C-fibers [106, 107]. Brief heat pain stimulation activates both Aδ (first pain) and C-fibers (second pain) [47]. As shown by both noxious electrical [48] and heat [108] stimulation of C-fibers, second pain *increases* by repeated stimulation with a frequency above 0.3/sec. However, first pain is *reduced* by repeated contact heat pain stimulation with an interstimuli interval of 80 sec or less [108]. Adaptation of first pain may be partly modified by peripheral effects, as shown by a study where suppression did not occur when alternating the probe site between stimulations, whereas temporal summation of second pain occurred even when the location of the probe was changed [109, 110]. Pain scores by repeated and prolonged/constant painful stimulation are correlated, and probably both share the physiological process of temporal summation [111, 112]. Temporal summation is probably centrally mediated by increments in spinal nociceptive neurons [113, 114]. The increase may be due to

modulation from the PAG-RVM as these regions also show increased activity during repetitive heat pain stimulation [115].

We believe adaptation is a suitable term to describe a decrease in pain score during *tonic* stimulation. Several papers use the term and separate it from habituation, a term used to describe a decline in response to *phasic* stimulation [116-119], although the literature is inconsistent [120, 121]. Adaptation and habituation are not defined by the IASP Taxonomy [42], but the characteristics of habituation are defined by Rankin et al. [122] as a behavioral response decrement by repeated stimulation that is not attributed to peripheral adaptation/fatigue. The mechanism of habituation is thought to be mediated by increased anti-nociceptive activity [123].

Migraine pathophysiology

The pathogenesis of migraine is complex and remains incompletely understood. Previously, the leading theory was that arterial dilatation caused migraine headache, but recent evidence suggests that vasodilatation is neither necessary, nor sufficient in migraine [124, 125]. It is now widely accepted that migraine should be viewed as a complex neurological disorder that affects cortical, subcortical and brainstem areas involved in autonomic, cognitive and sensory functions [126, 127]. Nevertheless, endothelial vascular cells may play a role in migraine pathophysiology through the release of numerous mediators that may activate nociceptors and contribute to migraine pain [128].

Migraine genetics

Migraine has long been considered to be a heritable disorder [129]. Relatives of migraineurs have increased risk of developing migraine [130]. Twin studies suggest that one-half of the liability to migraine is attributable to genetic factors [131-134]. Genetic studies have shown subtle and diverse genetic signatures in migraine that may affect ion channels [135, 136]. The genes associated with familial hemiplegic migraine, a rare hereditary form of migraine, are all involved in the control of brain excitability [137]. Genome-wide association studies in typical migraine have implicated both the cold sensor TRPM8 [138] and the pain sensor TRPA1 [139]. However, most identified susceptibility loci are associated with vascular and smooth muscle tissues, lending support to vascular contribution in migraine pathophysiology [140, 141]. Single genetic hits are associated with small odds ratios for disease risk [141], and the susceptibility of migraine is likely a sum of several genetic factors and environmental influences [142].

The anatomy of migraine pain

A migraine headache may depend on the activation and sensitization of the trigeminovascular pain pathway and dysfunction of central nervous system structures involved in modulation of excitability and pain [143-145]. Activation of nociceptive neurons innervating pial, arachnoid and dural blood vessels, and large cerebral arteries and sinuses are believed to give rise to the headache during migraine attacks [146]. They originate in the trigeminal ganglion and upper cervical dorsal root ganglia and terminate in the spinal trigeminal nucleus and upper cervical spinal cord (C1-3) in lamina I, II and V. Sensitization of meningeal nociceptors may be due to an acute sterile inflammation [147, 148]. Indeed, aseptic meningitis shares many of the same symptoms as migraine, like throbbing headache, nausea, vomiting, and photophobia [149]. Application of inflammatory soup to the rat dura has shown enhanced thalamic, hypothalamic, hippocampal, and S1 responses to mechanical stimulation of the face [150]. However, several studies have suggested that neurogenic inflammation plays a minor part in the migraine disease process [137].

The second-order neurons project to many different supramedullar brain structures involved in sensory, affective, endocrine and autonomic functions [144]. Most neurons are relayed in thalamus and synapses to third-order thalamocortical projection neurons. Altered oscillations measured by electroencephalography (EEG) that may indicate “thalamocortical dysrhythmia” [151-153], altered thalamic connectivity measured by resting-state fMRI [154, 155] and morphological abnormalities of thalamic nuclei [156] have been implicated in migraine, alterations that may be due to a complexity of factors. Different neurotransmitters may influence the modulation of nociceptive signals from the thalamus to the cortex, e.g., inputs from cortical, hypothalamic, brainstem, spinal, and intrathalamic nuclei, with axons containing glutamate, GABA, dopamine, noradrenaline, serotonin, histamine, orexin, and melanin-concentrating hormone [157].

How does a centrally mediated sensitization activate primarily trigeminal nociceptive neurons and not generate pain throughout the body? Meningeal sensory neurons do not seem to have unique properties or qualitative differences compared to nociceptive neurons in other tissues [158]. However, descending modulation of pain from PAG-RVM may affect the trigeminal and spinal dorsal horn differently, as suggested by predominantly GABAergic projections to postsynaptic neurons in the spinal dorsal horn but only modestly GABAergic projections to the trigeminal dorsal horn [159]. Also, RNA sequencing of dorsal root ganglia nerves and trigeminal ganglia nerves has identified several uniquely expressed genes in either ganglia [160]. These cellular and molecular differences may be of importance, but also the unusual

properties of the intracranial space, e.g., uniquely sensitive to changes in pressure and being a chemically privileged site [158].

Aura

Leão's cortical spreading depression (CSD), a wave of electrophysiological hyperactivity followed by a wave of inhibition, may be the mechanism underlying migraine aura [161-163]. Recent findings suggest that CSD could be the trigger of intracranial neurogenic inflammation, which may activate trigeminal afferents, including "silent" nociceptors, causing migraine [164-166], as shown by CSD-evoked mechanical sensitization of meningeal afferents in rats [167]. FMRI and magnetoencephalography findings in humans suggest that an event such as CSD may generate aura [162, 168]. CSD may be present in migraine without aura as well, but only affect "silent" cortical areas, although CSD-like events in migraine without aura have not been shown [144]. Migraineurs may have abnormal cortical excitatory and inhibitory balance, increasing the susceptibility for CSD [143]. However, although CSD may be the cause of aura, it is unlikely to be the primary migraine generator because it does not explain the preictal symptoms that may be present hours to days before a headache.

The preictal phase

Many of the suggested migraine triggers may influence central nervous modulation of sensory activity and distort the central excitatory and inhibitory balance in neuronal pathways. This imbalance may lead to migraine attacks in susceptible subjects [137]. For instance, migraineurs reported more pain compared to controls in the neck and trigeminal area after cognitive stress, a possible migraine trigger [169]. Reported subjective stress-symptoms probably changes over the days before a migraine attack [170-172], but whether such symptoms are triggers or a manifestation of the impending attack is not clear [171]. Subjective distress may reflect one of several cognitive, emotional or physiological homeostatic stressors, such as altered sleep and hormonal fluctuations, which may influence pain processing and increase the susceptibility of an attack [127].

Functional imaging studies have shown increased activation of the spinal trigeminal nuclei and hypothalamus in the preictal phase, followed by activation of several brain stem areas [173-175]. Preictal hypothalamic activation may explain many of the preictal symptoms, including concentration problems, tiredness, and irritability [142, 176]. Also, it may provide some insight into why migraine is commonly triggered by a change in homeostasis [177]. Neuroimaging studies also show activation in the dorsal pons, and the generation of a migraine attack is probably subject to alterations in several subcortical and

brainstem areas [178, 179]. Denuelle et al. [180] demonstrated hypothalamic, midbrain, and pons activation during spontaneous attacks. Accordingly, hypothalamic activity may be important for the non-headache symptoms present before, during and after the headache.

Classification of migraine pain

Migraine pain is not easily classifiable [181, 182]. Neurogenic inflammation may activate nociceptors, but the pain does not arise from actual or threatened damage to non-neural tissue and "nociceptive pain" may not be appropriate. Studies have not shown a lesion attributed to migraine (besides the white matter lesions as indicated by some studies [183, 184]), but migraine may be defined as a disease of the somatosensory nervous system and classified as "neuropathic pain." Findings of altered pain thresholds and habituation further support this classification. On the other hand, pending a better understanding of the migraine pathophysiology, "nociplastic pain" may be the term best suited by comprising findings of altered nociceptive function without a specific functional or anatomical alteration causing the pain.

Experimental pain and migraine

Several studies have investigated responses to experimental pain in migraine. Most of these studies compared responses from migraineurs and controls, but some also compared migraine phases, migraineurs with or without aura, or episodic and chronic migraine. Experimental pain seems to be associated with migraine frequency, as discussed below, but pain responses do not appear to differ between migraineurs with and without aura [4, 185-187].

Allodynia

Allodynia appears to be an important clinical correlate for altered pain processing in migraine. When evaluated by questionnaire, about 50-70% of migraineurs report allodynia during headache, and allodynia is associated with frequency and severity of migraine [188-191]. Subjects with chronic/transformed migraine (more days with than without headache) seem to have more allodynia, and lower pain thresholds than episodic migraineurs, indicating a relationship between altered pain perception and headache frequency [191-195]. However, pain threshold and tolerance of temporal artery stress did not differ between chronic migraineurs and controls [196, 197]. Migraineurs that have interictal allodynia may have hyper-excitable brainstem interneurons, as shown by enhanced non-nociceptive blink reflex recovery cycle [198]. In rats, application of inflammatory mediators to the dura

elicits facial and hindpaw allodynia within three hours, with increased activation of RVM "ON" cells and decreased activation of "OFF" cells [199].

A case report showed that, during a migraine attack, allodynia started on the same side of the head as the headache, then spread to the other side of the head, and finally to the arm with a progressive increase in magnitude [200]. The authors suggested that this represented activation of peripheral nociceptive neurons, followed by sensitization of second order spinothalamic neurons and lastly third order thalamocortical neurons [200]. Interestingly, migraineurs with extracephalic allodynia during attacks show greater blood oxygenation level-dependent signal activation in the posterior thalamus by brush or heat stimulation when stimulated during attack compared to when they were free from migraine and allodynia [201]. In one study, at least one of heat, cold or mechanical ipsilateral trigeminal allodynia was present in 79% of migraineurs 3-4 hours into an attack [202]. Only five of those 33 subjects had ipsilateral trigeminal allodynia without contralateral or non-trigeminal allodynia, and two had contralateral but no ipsilateral allodynia [202], thus not providing any clear evidence of sequential activation of first to second to third-order trigeminal neurons.

Cortical responses to painful stimulation

As summarized in a review by Schwedt et al. [203], fMRI-responses to painful stimulation in migraineurs have been extensively researched [175, 201, 204-213]. Multiple brain regions, involved in both discriminative, affective, cognitive, and modulatory aspects of pain, have shown increased or decreased activation [203]. For instance, a study of contact heat evoked potential stimulation of the maxillary skin showed greater event-related fMRI-activation of ACC and less activation of S2 in migraineurs compared to controls. The authors suggested that this represented increased antinociceptive activity as a compensatory mechanism to modulate pain perception at the same intensity in migraineurs compared to controls [211]. Another study demonstrated that 20 minutes of daily painful stimulation delivered over eight days increased the fMRI-activity level in prefrontal cortex and ACC in controls. The effect was the opposite in migraineurs, suggesting altered modulation mechanisms [204]. Pain processing changes before the headache starts, as shown by increased activation in the trigeminal nuclei by painful intranasal ammonia stimulation in the preictal phase, compared to both the interictal and ictal phase [175]. Also, photic hypersensitivity in the preictal phase indicates increased activation of the visual cortex [214]. To summarize, the migraine brain seems to process nociceptive stimuli different from controls between attacks, and the activation changes *before* headache starts, supporting that the migraine generator is in the brain.

The migraine cycle

Studies have shown reduced pain thresholds to either heat, cold or mechanical stimulation [202, 209], decreased pain thresholds tested by laser-stimulation [215] and facilitated nociceptive specific blink reflex responses [216] during attack compared to between attacks. Reduced HPT and CPT may be present in the preictal phase as well, as shown in a longitudinal study [4]. Another study with between-subjects comparisons found no difference in preictal (and postictal) heat, cold and pressure pain thresholds compared to interictal thresholds [217]. These two studies differed in the definition of the preictal phase, as Sand et al. [4] applied a 24-hour limit while Engstrøm et al. [217] applied a 48-hour limit. Interestingly, Sand et al. [4] found no difference with a 72-hour limit, indicating that different limits may be the source of the discrepant results. A correlation between heat pain thresholds and time to next attack has been shown, that is, thresholds decreased closer to next attack [218]. Average pain scores to painful intranasal ammonia stimulation were not different between the interictal, preictal and ictal phase in one study [175], and between the interictal and ictal phase, and controls in another study [213]. However, the change in pain scores during 15 consecutive stimuli increased in the interictal phase compared to habituation in controls, while pain scores were stable in the ictal phase [213]. Long-term habituation (the difference between day one and eight after daily stimulation) of pain scores to intranasal ammonia stimulation was present in both migraineurs in the interictal phase and controls with no group differences [204]. To summarize, some studies show decreased pain thresholds or increased response to painful stimuli during or close to the attack, but the results vary, and the effect sizes are small. A larger longitudinal study may show more defined differences if present.

Migraineurs in the interictal phase compared to controls

Studies that compared migraineurs in the interictal phase and controls have shown reduced thresholds and tolerance to heat and cold pain [217-219], decreased visceral pain threshold shown by over-distension of forearm veins [220], and low pain thresholds to light and sound stimuli that further declined during the attack [185, 186]. However, other studies have shown no difference in pain threshold or tolerance to the cold pressor test [221], no difference in pain thresholds for heat [102] and electrical [102, 187] stimulation, no pain score differences by suprathreshold heat pain stimulation [209, 211], but slightly reduced mechanical (von Frey) pain thresholds [102]. Migraineurs might have increased pericranial muscle tenderness [222, 223] and decreased pericranial pressure pain thresholds [197, 223-227]. However, other studies found no difference in pericranial muscle tenderness [196] or pressure pain thresholds [194, 217, 228, 229]. One study found increased electrical pain-related evoked potentials and

nociceptive blink reflexes [195], but conflicting results exist [230]. Fernández-de-las-Peñas et al. [223] argue that the conflicting results may be due to not controlling for headache laterality, as thresholds were decreased only on the symptomatic side in their study. However, a follow-up study by the same authors did not find side differences in strictly unilateral migraine [225]. Temporal summation of pain has been shown to be increased in migraineurs compared to controls for repeated electrical and mechanical stimuli, but not for tonic heat pain stimulation [102]. However, another study showed no difference in pain score changes between two trains of painful electrical stimulation [187]. In conclusion, no experimental pain test has so far proved reliable in distinguishing between persons with and without migraine. However, in general, migraineurs seems to be slightly more sensitive to painful stimuli between attacks compared to controls.

Habituation of laser-evoked potentials

Habituation of evoked responses has been extensively investigated in migraineurs between and during attack [13, 231-233]. Most studies have investigated cortical responses to innocuous stimuli. However, evoked potentials by noxious stimuli might be a more clinically relevant measure in migraine. Carmon et al. [234] demonstrated that a brief laser pulse could elicit cortical potentials that correlated with subjective sensation of pain [235, 236]. Noxious stimulation with laser selectively activates both Aδ and C-nociceptive neurons [234, 237-240]. Aδ-stimulation evokes cortical components, N1, N2, and P2, with latencies of about 170, 250, and 390 ms, respectively [241]. Most studies agree in that N1 originates in the operculo-insular cortex (S2 and DPINS) [242-248], as confirmed by intracortical recordings [249, 250]. Some studies suggest that the nociceptive response is generated mainly in DPINS and to a less extent in S2 [87, 251, 252]. Several studies also show contribution from S1 with both extra- [244, 247, 253-255] and intracortical [256, 257] recordings. Thus, it may be more than one source contributing to the N1, and laser-evoked potentials (LEP) from different sources may even be generated by parallel spinal pathways [258-260]. Virtually all studies agree in that ACC is the main source of the vertex N2P2 [238, 254, 261], as confirmed by intracortical recordings [262].

Several studies have demonstrated less prominent, or a lack of, habituation of evoked potentials in migraineurs in the interictal phase compared to controls for various modalities, but the findings are not easily reproducible for the most studied visual stimulus [6, 10, 13, 263]. Migraineurs in the interictal phase may have deficient habituation of both the N1 [264, 265] and the N2P2-amplitude [265-269], but the results lack independent replication. Similar results have also been observed in painful radiculopathy [270], fibromyalgia [271] and cardiac syndrome X [272]. In contrast to other evoked potentials, the

deficient habituation of LEPs may also be present during the ictal phase [268]. In the interictal phase, lack of habituation of the nociceptive blink reflex has been shown [273-275], with a tendency towards normalization during the migraine attack [275]. Habituation of LEPs in migraineurs has mainly been investigated by one group. Thus, the finding of deficient interictal habituation needs independent replication. Also, cyclical variations of LEP amplitudes and habituation are yet to be explored.

Transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) is a widely used tool to quantify the excitability of M1. Stimulation of M1 with TMS activates contralateral muscles corresponding to the stimulated brain area. The minimum stimulation power needed to create a motor evoked potential measured by electromyography in the muscle is defined as the motor threshold, a measure of the cortical excitability. Several stimulation protocols that measure specific subcomponents contributing to the cortical excitability have been developed, such as motor threshold, paired-pulse TMS and silent period [276].

TMS may alter the cortical excitability lasting beyond the stimulation session. This is achieved by applying repetitive trains of stimulation, known as repetitive TMS (rTMS). The frequency of the stimuli typically determines the effect on the cortical excitability, inhibition with stimulation at 1 Hz and facilitation with stimulation at 5 Hz and higher [277, 278]. However, the effect of rTMS may depend on the state of cortical excitability before stimulation, as shown by priming with transcranial direct current stimulation (tDCS) [279-281].

Repetitive TMS and experimental pain in healthy subjects

Several studies have shown an effect on experimental thermal pain thresholds of rTMS directed to various cortical areas in healthy subjects. One study compared the effect of 10 Hz rTMS over M1, S1, S2, and DLPFC. HPT on the face increased most and lasted longest after rTMS over S2 [282]. In men, S2 stimulation also elevated CPT [282]. A single-pulse TMS to S2 delivered 120 ms after a nociceptive laser stimulus reduced the pain intensity compared to TMS to S1 [283]. S2 tDCS did not alter experimental pain intensity [284] but reduced the objective pain-evoked activity in S2, as measured by magnetoencephalography [285]. However, simultaneous stimulation of S2 with pairs of TMS pulses and the dorsum of the hand with a laser did not alter laser pain thresholds [286]. Also, a small study applied 10 Hz rTMS with a double-cone coil to stimulate the DPINS and found no significant effect on thermal sensory and pain thresholds on the contralateral hand [287]. The stimulations used in these studies with

negative results differ and therefore do not disprove the possible effectiveness of high-frequency rTMS to S2 to alter pain perception.

It seems that high-frequency rTMS may be more effective than low-frequency rTMS to M1 in altering thermal pain thresholds and that the stimulation may increase CPT more efficiently compared to HPT [282, 288-291]. RTMS to M1 may also increase cool and warm detection thresholds (CDT and WDT, respectively) [282, 288-290, 292]. Subjective pain scores, associated with heat pain stimulation after capsaicin-induced hyperalgesia, decreased following 10 Hz rTMS to M1 [293]. One Hz rTMS to M1 decreased pain scores induced by capsaicin in one study [294], but *increased* pain scores and evoked potentials from laser stimulation in another study [295]. A recent study on the effects of 20 Hz rTMS to M1 on laser-induced pain demonstrated no difference between active or sham stimulation [296].

High-frequency rTMS to DLPFC increased HPT [297] and CPT [289, 291], and lowered heat pain ratings [298] in some studies, but WDT [282, 289], CDT [289], HPT [282, 289] or CPT [282] were unaltered in other studies. Low-frequency rTMS to DLPFC increased cold pressor tolerance during stimulation, but no long-term effects on the cold pressor threshold or tolerance, HPT, or pain pressure threshold were detected [299].

The results are heterogeneous. Only one study has compared the effect of rTMS on pain thresholds between several cortical sites, rendering S2 the most promising target [282]. In general, high-frequency rTMS seems to be more efficient in altering experimental pain compared to low-frequency rTMS.

TMS in the treatment of migraine

A recent review concludes that the studies so far provide low or very low-quality evidence on the effect of TMS on pain control in migraine [300]. Occipital single-pulse TMS has been shown to have some effect in the acute treatment of migraine with aura [301, 302], possibly by disrupting the propagation of CSD [303]. Results from studies of the therapeutic effect of rTMS to M1 or dorsolateral prefrontal cortex (DLPFC) are mixed [304]. One Hz rTMS over the vertex was not effective [305], 10 Hz rTMS to DLPFC in chronic migraine was inferior to sham stimulation [306], but was found to be effective in a smaller study [307], and in a recent more extensive study (without comparison to sham) [308]. Finally, 10 Hz rTMS to M1 may lower headache frequency and severity [309]. Thus, the available evidence on the clinical efficacy of TMS in migraine is not convincing.

Repetitive TMS and neurophysiological measures in migraineurs

RTMS may increase or decrease the cortical excitability depending on the excitability before stimulation [279, 280]. The effect of rTMS to M1 seems to differ between migraineurs and controls [310-315]. In short, studies suggest impaired baseline excitability in migraineurs shown by altered effects of high- and low-frequency rTMS compared to controls, and normalization by priming with rTMS or tDCS. Studies of rTMS to the occipital cortex have yielded similar conclusions [316-319], i.e., the different effects between migraineurs and controls were interpreted as reduced preactivation of the visual cortex in migraineurs [318]. Our group has also investigated the effect of occipital 10 Hz rTMS on visual evoked potentials [11]. We were unable to reproduce previous findings, except the finding of increased habituation by rTMS in migraineurs (with small 8' checks, not with large 65' checks). Coppola et al. [320] showed that the early burst of high-frequency oscillations embedded in the somatosensory evoked potentials, thought to be generated by thalamocortical afferents, was initially low in migraineurs, but increased by 10 Hz rTMS to M1. Thus, high-frequency rTMS was believed to normalize interictal thalamocortical dysfunction. Overall, these studies suggest altered cortical responsivity in migraineurs in the interictal phase.

RTMS-studies of cyclical alterations in migraine are scarce, but a study that applied 5 Hz rTMS to M1 reported increased preictal (compared to interictal) motor evoked potential responses and reduced ictal and postictal responses [321]. Also, the increased response was associated with low attack frequency, while the decreased response was related to high attack frequency [321]. Thus, Cosentino et al. [321] suggested that fluctuations in cortical homeostatic mechanisms could underline the migraine attack recurrence.

As far as we know, only one study has investigated the effect of rTMS on experimental pain in migraineurs. That study showed reduced LEP amplitude over vertex after 5 Hz rTMS to M1, but the effect did not differ from sham stimulation, and the pain scores were unaffected [322]. To summarize, rTMS may alter both detection thresholds, pain thresholds, and pain scores in healthy subjects, S2 is a promising and more pain-specific target than M1 and DLFPC, and rTMS has shown different effects in migraineurs compared to controls, but little is known about the effect of rTMS on cortical pain modulation in migraineurs. It was accordingly appropriate to proceed with more precisely targeted navigated rTMS-studies of S2 on pain-physiology measures in migraine.

Aims

Several studies have argued in favor of longitudinal examinations to explore the migraine periodicity in detail [118, 323, 324]. Most migraine studies have investigated the interictal or ictal phase, but the preictal phase has received increasing attention in search of the pathophysiological processes that culminate with a migraine headache [14, 31-33, 38, 142, 173, 174, 325-331].

Paper II and III examine cyclic changes in experimental pain with a blinded, longitudinal design, which enabled us to characterize pain thresholds, pain scores and pain evoked responses thoroughly throughout the migraine cycle. Specifically, the study design yielded a substantial number of preictal measurements.

Paper I examines the effect rTMS to S2 may have on pain thresholds and pain scores in migraineurs in the interictal phase compared to controls. Most of the migraine rTMS studies have stimulated M1, and some have stimulated DLPFC or occipital cortex, but none has examined the effect of S2-stimulation. Keeping in mind that S2 shows decreased activation to pain stimulation in migraineurs [211, 332] and that rTMS to S2 was proven to be most effective in increasing pain thresholds in controls [282], investigating the effect of rTMS to S2 in migraineurs is of interest. Also, alteration of nociception is arguably a more relevant measure compared to motor cortex excitability.

The main aim of the studies was to investigate changes in experimental pain measures in migraineurs, both between hypothesized shifts in homeostasis by migraine phase and by external modulation of the cortical excitability by rTMS.

Our primary aims within each study were:

Paper I: To investigate the effect of rTMS to S2 on thermal pain thresholds and heat pain scores in migraineurs in the interictal phase, and compare the effect to controls.

Paper II: To investigate migraine periodicity of thermal pain thresholds and suprathreshold heat pain scores.

Paper III: To investigate migraine periodicity of LEP-amplitudes and habituation.

Our secondary aims were:

Paper I: To study the effect of rTMS to S2 on non-pain-related thermal detection thresholds in order to see if responses really are pain-specific.

Paper II: To compare pain thresholds and pain scores in migraineurs in the interictal phase and controls.

Paper III: To investigate the effect of aura, headache laterality, years lived with migraine, pain scores, and number of days to next attack on LEPs, and to confirm previously reported deficient LEP-habituation in the interictal phase compared to controls.

Methods

The papers in this thesis present data collected over two periods. Paper I presents data from 2010 and Paper II and III present data collected in 2012. The investigators were blinded to diagnosis (or migraine phase in the second to fourth examinations of the 2012 study) during the examinations in both studies. Migraineurs kept a headache-diary for four weeks before and four weeks after examinations.

The Regional Committees for Medical and Health Research Ethics approved both study protocols. All subjects gave their written informed consent. Both migraineurs and controls received compensation to cover expenses associated with participation. The inclusion process, demographical data, methods, and statistics are described in detail in the papers, but a brief synopsis follows.

Subjects

In the first study, we recruited 43 migraineurs and 34 headache-free controls. They responded to advertisement within our university and were thus mostly students or employees at NTNU. Forty-nine migraineurs and 31 controls participated in the second study. We recruited them by advertisement in the local newspaper, hospital, and university. Neurologists confirmed the migraine diagnosis. Participants were between 18 – 65 years. We included migraineurs with two to six migraine attacks and no more than ten days with migraine per month. They could use symptomatic, but not prophylactic migraine treatment. Migraineurs could not have more than seven days with tension-type headache per month. Controls could not have headache more than once a month. If they occasionally had a headache, we asked if they experienced the headache as painful, used abortive medication, and had consulted a physician regarding their headache. If they answered yes to more than one of these questions, they were

excluded. Neither could have diseases that might influence the results, or use drugs that could influence neuronal, vascular or muscular function. A list of exclusion criteria can be found in the papers.

Table 3 *Demographic and clinical data after exclusions.*

	Study 1, Paper I		Study 2, Paper II and III	
	Migraineurs (*n* = 26)	Controls (*n* = 31)	Migraineurs (*n* = 49)	Controls (*n* = 31[d])
Age	27 ± 8 [20-51]	30 ± 10 [19-56]	40 ± 10 [19-62]	38 ± 12 [21-59]
BMI	24 ± 6	24 ± 6	26 ± 3	25 ± 3
Women	23/88%	26/84%	41/84%	26/84%
Days since 1st day of last menstruation	17 ± 18	18 ± 13	17 ± 12	19 ± 10
MwoA, MA+MwoA, MA	15/58%, 4/15%, 7/27%	NA	27/55%, 18/37%, 4/8%	NA
Years with headache	13 ± 8 [2-34]	NA	21 ± 9 [1-40]	NA
Migraine days/month [a]	1:13, 2:12, 3:1, 4:0	NA	1:14, 2:30, 3:5, 4:0	NA
Migraine intensity [b]	1:1, 2:8, 3:17, 4:0	NA	1:2, 2:20, 3:27, 4:0	NA
Headache duration [c]	11 ± 14 [1-60]	NA	16 ± 21 [0.5-72]	NA

[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 (hours) of an attack with or without the use of symptomatic medication.

[d] We included 30 controls in Paper III because we were unable to obtain reliable LEP-measurements in one control. Data displayed as mean ± SD [range] or n/%. MwoA: migraine without aura. MA+MwoA: some attacks with and some without aura (both diagnoses according to ICHD-3 Beta [30]. MA: migraine with aura (in 100 % of attacks). NA: not applicable.

We excluded nine subjects from the analysis in the first study (paper I): six due to dysfunctional equipment, two due to sleepiness, and one because we were unable to determine the resting motor threshold. Eleven migraineurs were excluded from the statistical analyses because they were not classified as interictal. In total, we analyzed 26 migraineurs in the interictal phase and 31 controls. One migraineur withdrew consent in the second study (Paper II and III), and three did not meet for all four examinations. In total, 49 migraineurs completed 190 examinations and 31 controls completed one examination each (we were unable to obtain reliable LEP-measurements in one control who were excluded from analyses in paper III). Figure 1 in Paper II shows a flowchart of inclusions and exclusions for the second study. Figure 2 in Paper II shows the distribution of phase combinations among migraineurs for the second study.

Study design

Both migraineurs and controls met to one examination each in the first study. The examination consisted of determination of resting motor threshold by navigated TMS (defined as the lowest stimulator intensity needed to elicit motor evoked potentials with amplitudes of at least 50 µV in half of ten consecutive trials), baseline measurement of thermal detection and pain thresholds and heat pain scores, and two sessions of rTMS (one active and one sham) followed by new threshold and score assessments. In the second study, controls met to one examination while migraineurs met to four examinations each with approximately one week between sessions. Thus, we measured thermal pain thresholds, heat pain scores, and LEPs once in controls and four times in migraineurs. Migraine diaries categorized sessions as interictal, preictal (less than one day before the attack), ictal or postictal (less than one day after the attack). The procedures summarized below are described in more detail in the papers.

Thermal thresholds

We obtained thermal detection and pain thresholds with the method of limits. We used a Peltier thermode and measured thresholds on the thenar eminence on the hand and the forehead slightly lateral to the midline just above the orbita. Baseline temperature was 32 °C, and the temperature increased or decreased by 1 °C/s. To determine detection thresholds, we instructed the participants to press a button as soon as they perceived a change in temperature. To determine pain thresholds, we instructed them to press a button when the stimulus was perceived as painful. We measured each threshold four times on each site with random 4-6 seconds inter-stimuli intervals.

We obtained CDT, CPT, WDT, and HPT, in that order, on the hand before the forehead in the first study. Thresholds were determined three times, once before rTMS, once immediately after the first session with either real or sham rTMS, and lastly after the second session with rTMS. We obtained only pain thresholds, not detection thresholds, in the second study.

Heat pain scores

We determined the individual temperature that gave a pain corresponding to approximately six on a numerical rating scale (NRS) ranging from zero = “no pain” to ten = “unbearable pain.” This temperature was first applied on the forearm for 30 seconds while the participants continuously rated the experienced pain verbally throughout the stimulation, and later the procedure was repeated on the

temple. The pain score reported after 30 seconds was stored for analysis in the first study, while the last reported pain score at 0, 10, 20 and 30 seconds was stored for analysis in the second study.

Laser-evoked potentials

LEPs were obtained by stimulation on the dorsum of the right hand with an Nd:YAP laser. We recorded two blocks with an average of 21 consecutive stimulations with inter-stimuli intervals of 6-10 seconds. Two main analysis channels were preselected, Cz referred to nose and T3 referred to Fz (10-20 system). We stimulated with a laser intensity that invoked a sharp painful pinprick, and subjects scored the perceived pain verbally with a numerical rating scale ranging from zero to ten. The N1 and the N2P2 LEP-components were assessed, N1 at the contralateral temporal electrode (T3) and N2P2 at the vertex (Cz).

Repetitive transcranial magnetic stimulation

Navigated TMS and rTMS was only applied in the first study. We started with a mapping procedure to identify the site on M1 producing the most significant and reproducible motor evoked potential recorded from the abductor pollicis brevis muscle. The chosen site was used to measure RMT, defined as the lowest stimulator intensity needed to elicit motor evoked potentials of at least 50 μV in half of ten trials.

RTMS with 900 stimuli at intensity 90% of RMT was delivered over the S2 area (Figure 1). We applied high-frequency (10 Hz) rTMS. One session lasted for 4 minutes and 20 seconds and constituted of 18 trains of 50 stimulations with 10 seconds between-trains intervals, comparable to the protocol in a previous migraine study [318]. In addition to the real rTMS, we applied a sham stimulation with the coil tilted 90 degrees with the lateral part of the coil touching the same area as during real rTMS. The order of stimulation was randomized.

Data analysis

We applied multilevel linear regression to analyze the outcome variables in all three papers. In Paper I, we mainly analyzed the linear effect of the interaction between stimulation (baseline, sham, and rTMS) and group (migraineurs in the interictal phase and controls) on the outcome variables (CDT, WDT, CPT, HPT and pain scores). Thus, a significant interaction effect represents different responsivity to rTMS between controls and migraineurs. We have added additional analyses of preictal recordings from Study 1 (Appendix). In Paper II, the primary analyses were of the linear effect of the interaction between phase

(interictal, preictal, ictal and postictal) and site (head and hand) on the outcome variables (CPT, HPT, and heat pain scores). Similarly, in Paper III, we mainly analyzed the linear effect of the interaction between phase and habituation (the difference between first and second block) on the outcome variables (N1, N2P2, and LEP pain scores). Also, interictal recordings from the first day were compared to controls in both Paper II and III.

Synopsis of main results

Migraineurs in the interictal phase compared to controls

RTMS to S2 increased pain thresholds in controls compared to migraineurs (Paper I). The effect was present compared to both sham stimulation and baseline measurements, although the group-difference in rTMS-effect on CPT was not significant compared to baseline. RTMS decreased heat pain scores in both groups compared to baseline measurements. Hand CDT increased after rTMS in both groups.

Migraineurs had lower hand CPT than controls, interpreted as suballodynia in migraineurs (Paper II). LEPs showed habitation of N2P2, but no significant habitation of N1 in both migraineurs and controls (Paper III). The suprathreshold heat stimuli needed to elicit a pain score of six was lower in migraineurs in Paper I, but not significantly different in Paper II.

Migraine phases

Pain scores at the beginning of the 30 seconds stimulation period were lower in the preictal phase at both sites (forearm and temple). Also, pain scores decreased less from zero to 20 and 30 seconds preictally. Supplementary analyses from Study 1 also showed preictal hypoalgesia, demonstrated by increased trigeminal CPT and reduced trigeminal heat pain scores after 30 seconds in a small preictal subgroup (Appendix). We interpreted these findings as preictal hypoalgesia and reduced central adaptation.

Forehead CPT decreased in the ictal phase compared to both the interictal, preictal and postictal phases, interpreted as ictal trigeminal cold suballodynia (Paper II). Overall heat pain scores at the temple increased in the ictal phase, interpreted as ictal trigeminal heat hyperalgesia.

Both N1 and N2P2-amplitudes decreased from the first to the second block in the interictal phase, that is, habituation was present (Paper III). A post-hoc contrast showed deficient ictal habituation of N1.

Correlation with clinical factors

Neither pain thresholds, heat pain scores nor LEPs showed significant differences between migraineurs with and without aura. Headache laterality did not significantly affect pain thresholds, heat pain scores or LEPs, although subjects with a bilateral headache seemed to have less N2P2-habituation in the postictal phase. Also, preictal N2P2-habituation decreased with increasing years lived with migraine.

Methods discussion

Context information may have a significant impact on results in pain studies [333]. We applied procedures with precise and standardized instructions, but most importantly, the examiners were blinded to diagnosis (and phase). Blinding does not guarantee equality in instructions but contributes to making any differences random. The quality of blinding in previous neurophysiological migraine studies is variable and may be a significant factor in the variety of previous findings [334].

The number of ictal and postictal recordings in Paper II and III was low relative to interictal recordings. Power is based on the smallest sample size, so an alternative design such as asking the patients to present for a test session during an attack might be a better way of collecting a sufficient sample size for the interictal-ictal (and postictal) comparisons in future studies. However, we had no available around the clock physician-resources to handle randomly incoming migraineurs in attack. Also, controlling for other factors, e.g., time of day, blinding of phase and anticipation, would be more difficult. Finally, our goal was also to investigate the important preictal phase. Hence we had to choose random recordings with diary-based classification. The multilevel analyses can handle skewed groups well and enabled us to use all the available data with greater flexibility and to account for within-subject and within-day correlations properly [335].

Thermal thresholds

A quantitative sensory testing (QST) protocol is considered a useful method for psychophysical assessment of sensory detection and pain perception [336]. The thermal part of a QST-protocol is particularly helpful in diagnosing small fiber neuropathy, mostly as hypoesthesia and hypoalgesia. However, it can also document allodynia or hyperalgesia, a phenomenon that may imply peripheral or central sensitization [337]. Traditional threshold testing with the method of limits cannot wholly

differentiate between the sources of habituation/sensitization effects. We applied prolonged, painful heat stimulation in the second study to investigate the possible central sensitization better. If sensitization also occurs when a non-cephalic region is stimulated, one can probably address this effect to a central mechanism. We applied a relative intense NRS-6 stimulus. Although adaptation to repeated or tonic low-intensity stimulation on the same site may be of either peripheral or central origin, temporal summation to high-intensity phasic heat pain (or "intensification" to a similar tonic stimulus) is probably caused by central mechanisms [118]. More advanced protocols have been suggested to address this [110, 118], but we did not apply, e.g., spatial summation or cutaneous electrical stimulation in our present study. However, we stimulated at two different sites, one cephalic and one non-cephalic, and we interpreted either non-cephalic involvement or intensification of tonic NRS-6 pain as most likely representing a central mechanism.

Thermal testing with a Peltier thermode requires contact between the thermode and the skin, which may modulate perception by co-activation of mechanosensitive Aβ fibers [338, 339], but the influence is probably minimal by fast-adapting mechanoreceptors. The same investigator performed all measurements in our studies, applying the same grip and approximately the same pressure on the thermode to keep the activation of Aβ fibers constant between sessions.

The thermal thresholds measured by the method of limits are affected by reaction time. The stimulus continues to increase or decrease during the time it takes the participant to process information before pressing the button. An alternative is the method of levels, which is independent of the speed of response [340]. However, the method of levels is a more time-consuming method susceptible to errors from decreased attention by the subject [341]. We applied the method of limits with a commonly used slow rate of temperature change (1 °C/s). This rate will probably minimize any reaction time artifact, reduce the intra-individual variation of thresholds and minimize the incidence of ceiling effects in pain thresholds [342]. Also, we ran a practice session to make the participants accustomed to the test.

Cold pain threshold may be lower when tested after heat pain than in the reverse order [343], and detection thresholds may be higher (about 1 °C difference) if tested after thermal pain assessment [344]. We used the test order CDT, CPT, WDT and HPT in the first study. A better and more common order would be CDT, WDT, CPT, and HPT. Since we measured WDT after CPT, we might have slightly overestimated WDT. However, the outcome of interest was the change from baseline and not the baseline measure in and of itself, and we compared baseline measurements to controls who were subjected to the same stimulation order. Thus, we are confident the test order has not compromised the results.

Rather small intraindividual thermal detection threshold and HPT differences were found among healthy adults [344-350] while large standard deviations and coefficients of variation were observed for CPT, rendering it a less robust measure in repeated measures comparisons [346-348]. Numerous factors are known to influence QST, e.g., sex, age, attention, anxiety, motivation, prior instructions, and training and gender of the investigator [336]. Hence, we applied a standardized procedure to obtain reproducible results. The room was quiet with constant lightning (no windows), we read pre-written instructions to all subjects, and one examiner always performed the test in the same manner [341]. Migraineurs and controls were also matched by age and sex.

Heat pain scores

Subjects used NRS to report perceived pain. NRS has proved to be a reliable measure of subjective pain, comparable to both visual analog scale, verbal rating scale and faces pain scale [351]. The stimulation intensity (probe temperature) was individually determined to increase the number of subjects showing temporal summation, and reduce floor and ceiling responses [352, 353].

The session-to-session repeatability of pain scoring during tonic and phasic suprathreshold heat pain stimulation is acceptable on a group basis but may show considerable intraindividual variability [112], although other studies show good repeatability for phasic stimulation [354], and subjects' ratings at threshold [348].

We could not analyze early effects of rTMS on pain scores in the first study as we only stored NRS at 30 seconds. However, Koyama et al. [119] have shown a close relationship between scores after 30 seconds of noxious heat stimulation, peak scores, and mean score. Thus, the pain scores analyzed in Paper I probably reflects the change in the overall suprathreshold pain experience during 30 seconds of stimulation.

Laser-evoked potentials

We stimulated the hand only, and interpretation of the LEP-results is thus valid only for the more global pain function in migraine. Our aim was to study the generalized effects since LEP reflects activation of a large part of the bilateral cortical pain network and hand and cephalic LEP habituation seemingly are correlated [264-266, 268, 269]. Nevertheless, trigeminal stimulation is necessary to conclude about lateralized second order medullar afferent sensitization can be detected by LEP-abnormalities.

Type of laser, laser beam size, and stimulus duration vary considerably between studies. According to Treede et al. [239], pain thresholds are nearly constant when expressed as energy per unit of skin

area within 3–9 mm beam diameter. Due to its shorter wavelength, Nd:YAP lasers are not entirely comparable to CO2 lasers using longer stimulus durations. Hence, by trial we have previously observed that an 8-mm diameter produced painful pinprick sensations with 2-3 J intensity in most subjects, with less discomfort than 5 and 6 mm diameters that have higher energy densities, allowing reasonable intensities around 4.5 J for the stimulus target [355]. The diameter and durations are comparable to other researchers using the same type of laser [356, 357].

Finding the right stimulus intensity is an important issue, but somewhat underreported in the literature (e.g., not discussed in the SEP-guideline [358]). Our initial aim was to use fixed stimulus intensity equal to 1.5-2 times the threshold in healthy subjects [239, 261]. However, we observed that a fixed intensity did not always elicit pain in every healthy subject and, on the other hand, a few subjects could not tolerate this "mean fixed intensity" [355]. Hence, we, as suggested by others too [357, 359, 360], decided to use stimulus intensities based on individual thresholds to obtain reliable LEPs. We applied the same procedure as in a former study by our group [355], i.e., we chose the intensity corresponding to two times the pinprick threshold in most participants. Such individually defined tolerable stimuli tend to produce reliable LEP-responses [70].

Repetitive transcranial magnetic stimulation

RTMS is considered safe [361]. A seizure is the more severe TMS-related acute side-effect, but this is extremely rare [362]. Most safety studies have analyzed rTMS to M1, but rTMS to non-motor areas appears to be equally safe [363]. We applied rTMS according to guidelines [362] and did neither include subjects with known epilepsy nor subjects under treatment with drugs that potentially lower the seizure threshold. The most common side effects of rTMS are pain, discomfort and headache [361-366]. Twelve participants in the first study experienced one of these minor side effects during or shortly after stimulation.

We included a sham stimulation to control for unspecific effects in the first study. We randomized the order of presentation of stimulation, sham vs. active first, to control for order effects. It is hard to mimic the various sensations evoked by rTMS as it generates both sound and a "hammering" effect, and can activate peripheral nerves and muscles causing pain and discomfort. Several authors have tried to recreate those sensations by electrical stimulation during sham rTMS [367-370]. Although these procedures may generate a better sham, they are also complex and time-consuming. We chose to apply a simplified approach. Tilting the coil 90° gives the same contact area, sound and "hammering" sensation without affecting the cortex. We emphasized the importance of the coil position to generate a genuinely inactive sham. Tilting the coil 90° induces lower voltage-differences

in the brain compared to 45° [371], and touching the scalp with the lateral edge of one wing is better than with the front edge [372]. Hence, we are confident that the sham stimulation we applied did not produce a partially active stimulation. However, the lack of muscle activation and slight pain some experienced during real rTMS may obviously have compromised the blinding. Collection of specific data on how the stimulations were perceived and on blinding success would have made it possible to quantify the success of sham stimulation, but this has not been standard practice [373]. Although our sham-procedure was not perfect, we believe it to be a sufficient control of the placebo effect.

The use of live MRI-guided navigation enabled us to aim at S2 with high precision, and prevented unintended movement of the coil during stimulation. Even minor changes in coil location and orientation may have a significant effect on the outcome [374, 375]. Target location has been significantly improved using navigation systems considering individual brain anatomy [376, 377].

Gender, exercise, time of day, age, attention, priming, pharmacology, and genetics are factors known to influence the magnitude and direction of the effect of noninvasive brain stimulation [378]. We asked participants to avoid exercise, cigarettes and caffeine-containing beverages the morning before the examination. All examinations were done at the same time of day. Migraineurs and controls were matched for age and sex and did not use central nervous system active drugs. As a result, potential between-group external factor effects were minimized, and we interpreted the findings in Paper I as representing mainly pathophysiological differences between migraineurs and controls.

Data analysis

The multilevel analysis is an extension of ordinary linear regression that can account for dependencies in the data. Our outcome variables were clustered due to the repeated measurements within subjects and days. Variations of analysis of variance (ANOVA) is historically used to analyze this kind of data, but multilevel linear regression has several advantages compared to ANOVA [379]. Examples are handling of missing data, unbalanced numbers of repeats and three-level or higher hierarchies. The most prominent caveat with multilevel linear regression compared to ANOVA is its complexity, and the researcher should provide clear descriptions with justification of the chosen models. Modern statistical software have become more user-friendly, and several papers and books have made these methods readily accessible (e.g., [335, 379-382]).

Interpretation of results differs between multilevel models and ANOVA. Whereas ANOVA reports means and a p-value that says whether at least two groups or conditions have been sampled from

significantly different populations, the fixed effects reported in the multilevel models should be interpreted in the same way as results from ordinary linear regression. That is, a regression model reports only one mean (the intercept), and the differences between that mean and all other means, with p-values for each specific difference. Although we apply quite complex multilevel models in the papers with different variance-covariance matrices, different specification of random effects and slopes, and the inclusion of censored variables, the primary interests are in the fixed effects. Thus, for simplicity, one may concentrate on only the fixed part and interpret the results as one would with ordinary linear regression.

Several of the participants did not experience any pain before reaching the limit of 5 °C for the cold pain test. We knew that these values were below 5 °C but not by exactly how much. These responses were defined as "censored" [383]. Handling of censored responses have consequences on the results, especially when the number of censored responses are skewed between groups [384]. Similarly, the LEP-component N1 was too small to be distinguished from surrounding noise in some traces, and we presumed that the response was within the interval bounded by zero and the maximal noise within the N1-time frame (interval-censoring). Both types of censored responses can be incorporated in the multilevel models. "Missing" CPT-values have usually been imputed as the technical ceiling value, but this may underestimate group differences. Alternatively, exclusion of such missing values creates bias. Hence, the present handling of censored values in paper II and III is an advantage because it preserves all information in the dataset.

Discussion

The main finding in the first study was a significant increase in pain thresholds after rTMS to S2 in controls compared to migraineurs in the interictal phase. In the second study, we found preictal hypoalgesia and reduced adaptation by tonic heat stimulation of both forehead and hand, and ictal trigeminal heat hyperalgesia and cold suballodynia. Also, there was a subtle lack of habituation of the N1 LEP-amplitude in the ictal phase. We summarize our combined results with interpretations and possible mechanisms usable as hypotheses for future studies in Table 4 below. We found clear evidence from several measures, including rTMS, of a generalized central sensitization to thermal pain interictally. This state seems to be temporarily replaced by preictal hypoalgesia followed by a more locally dominant trigeminal increased ictal hyperalgesia. Actual pain score correlated by LEP-amplitude in migraine while slightly reduced LEP-habituation tended to reflect the ictal hyperalgesia that was most evident from threshold and suprathreshold pain measurements.

It should also be mentioned that the significant findings were few and the effect sizes were relatively small. On the other hand, the present findings may be an essential addition to existing theories and give guidance for future research.

Table 4 *Combined results for pain-measures with implications and possible mechanisms usable as hypotheses/recommendations for future studies.*

	Effect of rTMS	Group comparisons	Intraindividual comparison (vs. interictal phase)			
	Migraineurs[a] vs. controls	Migraineurs[a] vs. controls	Preictal	Ictal	Postictal	Combined results from actual pain measure
Cephalic and non-cephalic thermal pain thresholds	Lack of normal cold and heat hypoalgesia at cephalic and non-cephalic site.	Substantial hand cold suballodynia in migraineurs (Δ 4.4 °C).	No change.	Trigeminal cold suballodynia.	No change.	Interictal hand cold suballodynia with emerging trigeminal cold suballodynia in the attack phase.
Cephalic and non-cephalic suprathreshold tonic heat pain	Lower NRS-6 stimulation temperature at cephalic and non-cephalic site.	No group differences. Increase in pain score at cephalic and non-cephalic site towards the next attack.	Heat *hypo*algesia and reduced adaptation at cephalic and non-cephalic site.	Trigeminal heat hyperalgesia.	No change.	Increasing interictal generalized hyperalgesia, preictal generalized *hypo*algesia, ictal trigeminal hyperalgesia.
Non-cephalic LEP N1 (from S2 and DPINS)	NA	No group differences. No habituation. Amplitude correlates with pain score.	No change. Habituation present.	No change. Deficient habituation (post hoc).	No change. Deficient habituation (post hoc).	Pain score correlates with N1 first-block amplitude. N1 amplitude lack sensitivity to phase. Subtle lack of ictal and postictal habituation.
Non-cephalic LEP N2P2 (from ACC)	NA	No group differences. Habituation present. Amplitude correlates with pain score.	No change. Habituation present.	No change. Habituation present.	No change. Deficient habituation (post hoc).	Pain score correlates with N2P2 first-block amplitude. N2P2 amplitude lack sensitivity to phase. Subtle lack of postictal habituation.
Non-cephalic LEP pain scores	NA	No group differences. No change from first to last stimulation.	No change. Potentiation present.	No change. Potentiation present.	No change. Potentiation present.	Pain score potentiation as opposed to LEP habituation, invariant to phase.

Table 4 *(continued).*

	Effect of rTMS	Group comparisons	Intraindividual comparison (vs. interictal phase)			
	Migraineurs[a] vs. controls	Migraineurs[a] vs. controls	Preictal	Ictal	Postictal	Combined results for all pain measures
Main implication	Generalized reduced analgesic effect of rTMS to S2 in migraineurs. Generalized hyperalgesia (before rTMS).	Hand cold suballodynia and generalized subtle heat hyperalgesia that are more pronounced close to attack. LEP does not reflect migraine-specific physiology interictally but seems to reflect actual pain.	Transition from interictal hyperalgesia to preictal hypoalgesia. (No confirmation of previously reported generalized preictal HPT and CPT suballodynia [4]).	Trigeminal sensitization during attack evident in both the cold and heat domain, possibly related to decreased generalized N1 habituation (reduced ICI in S2/DPINS).	Normalization of ictal trigeminal sensitization. Persistence of a slight generalized habituation deficiency.	S2 may not be the best target for migraine TMS treatment. Transient preictal hypoalgesia, preceded and followed by hyperalgesia, most easily found with CPT, may be the essential cyclic pattern in many migraineurs. LEP- habituation may reflect phase-modulation better than LEP-amplitude.
Possible mechanisms and hypotheses for future studies	Reduced activation of central pain modulatory mechanisms in migraineurs. Central sensitization of thermal pain.	The suballodynia may reflect arousal-induced reduction of ICI. LEP may reflect perceived pain in migraine, fitting with reduced ICI in S2/DPINS and ACC.	Briefly decreased cortical activation that may be related to reduced arousal, increased ICI, and/or thalamocortical hypoexcitability leading to increased preictal OFF-cell activity.	Persistent central sensitization with local (trigeminal) exacerbation, caused by activation of peripheral neurons and induction of neurogenic inflammation.	Transition to the state of interictal generalized hyperalgesia.	LEP and perceived pain depend on cognitive states (e.g., arousal, vigilance, sleepiness). Brief phasic pain may elicit a different state than tonic pain, explaining why pain sometimes correlate with LEP amplitude as (reduced ICI?) and the opposite after sleep restriction (reduced descending inhibition?) [355].

rTMS transcranial magnetic stimulation, LEP laser-evoked potentials, S2 secondary sensory cortex, DPINS dorsal posterior insula, ACC anterior cingulate cortex, NRS-6 numerical pain rating score at 6/10, NA not applicable, ICI intracortical inhibition, HPT heat pain thresholds, CPT cold pain thresholds.

[a] Migraineurs in the interictal phase.

The interictal phase – comparisons with controls

Non-invasive brain stimulation of the secondary sensory cortex

The significant increase in pain thresholds after rTMS to S2 in controls is comparable to findings by Valmunen et al. [282]. We applied high-frequency rTMS, a protocol that usually induces an increase in cortical excitability [385]. In a chronic pain model in rats, electrical stimulation of S2 decreased the expression of c-Fos in the trigeminal nucleus caudalis neurons [386], an antinociceptive effect mainly mediated through the descending spinal serotonergic pathway [387]. In mechanically hypersensitive rats, the antinociceptive effect of S2 stimulation was associated with decreased response in RVM ON-cells to heat-evoked limb withdrawal [388]. Hence, one hypothesis may be that rTMS increased the excitability in S2 which increased the activity of the descending antinociceptive pathways (or decreased the pronociceptive activity by ON-cells) in the control group [282]. This effect was lacking in the migraine group, indicating that subtle cortical physiological changes in S2, or changes in descending pain modulation pathways affected by S2, may be of importance in migraine pathophysiology.

We demonstrated decreased pain scores after 30 seconds of suprathreshold heat stimulation in both migraineurs and controls by rTMS to S2. Experimental tonic pain may show a closer resemblance to clinical pain compared to pain thresholds. High-frequency rTMS to S2 has successfully decreased pain in chronic neuropathic orofacial pain [389]. A follow-up study showed that the decreased pain intensity by S2 stimulation was most likely due to a direct descending modulation effect and not mediated by improvement of comorbid psychiatric or sleep disturbances [390]. On the other hand, ten daily sessions of *inhibitory* (1 Hz) rTMS to the right S2 also showed an analgesic effect on pain intensity (in chronic visceral pain patients) [391]. The analgesic effect was present in both migraineurs and controls and has been shown in neuropathic and chronic pain, thus probably represents an antinociceptive effect unrelated to migraine. It remains to be determined whether this "normal" rTMS response changes before or during a migraine attack.

RTMS to S2 increased hand CDT in both migraineurs and controls. In comparison, inhibitory rTMS to S2 decreased perceived touch intensity and reduced S2 fMRI activity to touch in healthy subjects [392]. Imaging studies have demonstrated activity in S2 in response to innocuous thermal stimulation, with increased activity parallel to increase in temperature [68]. Activation by temperatures in the noxious range, however, seemed to raise activity in the insula [87]. Since the rTMS mainly affects the cortex only two centimeters in depth [393], it may not reach the medial cortical areas with a possible higher potential for pain modulation. However, we successfully lowered pain scores in both groups, and pain thresholds in controls, showing that rTMS with a

figure-of-eight coil may induce at least some analgesic effects. It remains to be seen whether deeper stimulation yields a more significant antinociceptive effect.

Thermal detection thresholds, pain thresholds, and pain scores

Hand CDT was lower in migraineurs in the interictal phase compared to controls in the first study, while hand CPT was lower in the second study. Neither WDT, HPT nor heat pain scores were different in migraineurs compared to controls. Cold sensitivity changes may accordingly be the more robust thermal feature that reflects a slightly generalized hypersensitivity in migraineurs. However, previous studies demonstrated altered thresholds for both cold and heat [217, 219], and stimulation temperature during suprathreshold heat stimulation was lower in migraineurs in the first study, implying heat hyperalgesia. Interestingly, several genome-wide association studies have identified a single-nucleotide polymorphism in a gene related to TRPM8, primarily a cool sensing receptor, associated with migraine compared to non-migraine headache and headache-free controls [394]. The role of TRPM8 in migraine is not clear [138], but a recent study has shown an analgesic effect of facial TRPM8 activation by suppression of TRPV1 [395], and meningeal TRPM8 activation caused allodynia in a study on rats [396]. Although speculative, an alteration in the TRPM8 receptor in migraineurs may alter both heat and cold detection and pain perception by complex interplay with pain receptors, like TRPV1 and TRPA1, increasing the susceptibility to trigger a migraine attack.

Laser-evoked potential amplitudes

The first-block LEP-amplitudes of both N1 and N2P2 were comparable to controls and correlated with pain scores, corresponding to previous findings [261, 397]. LEP does not seem to reflect migraine physiology, but actual pain. That is, LEP probably reflect the saliency of painful events rather than stimulus intensity per se [398-400]. Amplitudes of gamma band oscillations (power in the time-frequency domain) induced by laser also correspond to subjective pain intensity [401] but may be less affected by saliency compared to evoked potentials [402]. Tiemann et al. [403] demonstrated that gamma responses, as opposed to evoked potentials, were influenced only by stimulus intensity and not by placebo analgesia. They concluded that gamma oscillations might reflect nociception more closely than evoked potentials that may be more influenced by affective and evaluative processes [403]. Likewise, stimulus intensity during ten minutes of painful heat may be better represented by gamma oscillations in the somatosensory cortex, and pain intensity by gamma oscillations in prefrontal cortex [404]. The unaltered LEP-amplitudes and pain scores in interictal recordings compared to controls suggests normal pain processing in migraineurs, although more specific measures of pain processing capable of dissecting bottom-up and top-down processes, e.g., gamma oscillations, could prove to be useful.

Habituation

It is debated whether habituation of evoked potentials is deficient in migraineurs compared to controls [13, 232, 233, 405, 406]. The findings in Paper III agree with our previous findings of normal habituation of visual evoked potentials in migraineurs in the interictal phase [6, 10, 13]. Previous LEP-studies in migraine have suggested deficient habituation [265-269], but the findings in Paper III do not support those results. In fact, a recent study combined results from selected studies that previously had shown deficient habituation in migraineurs and calculated the diagnostic efficacy of visual and auditory evoked potentials [407], and the results showed no diagnostic utility in migraine [408, 409]. In our opinion, habituation of neither visual, auditory nor laser-pain evoked potentials in migraineurs are useful as a "diagnostic test" in the clinic. Habituation measurements have so far provided several interesting results and theories that are worthy of further exploration. However, due to the considerable variability in methods and findings from various groups, we still lack consistent and reproducible information about habituation in migraine.

Migraine periodicity

Preictal hypoalgesia and reduced central adaptation

The most surprising finding was the preictal hypoalgesia, as previous studies have shown preictal suballodynia and decreased HPT towards the next attack [4, 218]. However, studies have also found indications of reduced central nervous system activity in the period preceding a migraine attack, including decreased cortical activity during sleep [410, 411], decreased occipital excitability [412], decreased sleep latency [217], and thalamocortical hypoexcitability [327]. Supplementary analyses from Study 1 (Appendix) show trigeminal cold and heat hypoalgesia in a preictal subgroup, supporting the preictal finding in Paper II. A longitudinal study of post-movement beta event-related synchronization, possibly representing a cortical motor inhibitory mechanism [413], showed asymmetrical increased preictal synchronization and decreased ictal synchronization compared to interictal measurements [414]. The findings may indicate increased intracortical inhibition in the preictal phase followed by reduced ictal inhibition. Recently, high-frequency rTMS to M1 demonstrated a facilitatory effect on the motor evoked potential in the preictal phase, suggesting decreased cortical excitability compared to the other phases [321]. Cosentino et al. [321] postulated that the decreased cortical activity might raise the threshold for activation of inhibitory homeostatic mechanisms, lending the cortex more vulnerable to migraine triggers. Thus, our finding of reduced central adaptation during the prolonged stimulation may be due to decreased top-down anti-nociceptive regulation. On the other hand, the apparently reduced adaptation may be a consequence of the initial hypoalgesia. In fact, as evident by Figure 5 in Paper II [2], the pain scores at 30 seconds are equal in all phases, and the difference in slope may be due to different starting points. However, usually, low initial pain score promotes adaptation while high initial pain score

yields temporal summation during the first 30 seconds [118]. Nevertheless, the initial hypoalgesia suggests reduced cortical responsiveness, possibly due to thalamocortical hypoexcitability [327], and/or increased intracortical inhibition.

The transition from interictal to preictal phase

A previous study at our lab found preictal HPT and CPT suballodynia [4]. The suballodynia was present with a 24-hour preictal limit but not with a 72-hour limit. In Paper II, we did not reproduce these findings as HPT and CPT did not change from the interictal to the preictal phase, with neither a 24-hour nor a 72-hour limit. It is possible that preictal recordings were closer to the attack in our previous [4] than in the present study, although we found no association between pain thresholds and days to next attack. We need studies with a higher temporal resolution of migraine diaries to investigate a possible association between pain thresholds and hours to next attack. Pain scores to suprathreshold heat stimulation showed increasing generalized hyperalgesia during the interictal period with an abrupt and limited time of generalized hypoalgesia within the 24 hours preceding the headache. Preictal cortical alterations of pain processing may be more distinct by high-energy stimulation, i.e., suprathreshold stimulation compared to stimulation at thresholds, although neither LEP-amplitudes nor pain scores to laser stimulation showed altered preictal pain processing.

Nociceptive thresholds vary with the balance between ON- and OFF-cells in the PAG-RVM system [99]. The hypothalamus may be of importance for many of the preictal symptoms present before headache and has direct ascending and descending connections with structures involved in pain modulation [415]. Excessive OFF-cell discharge not only suppress nociception but also affects the hypothalamus causing symptoms like hunger, excessive urination, the need for sleep and tendency to avoid moving [415]. Nitroglycerin triggered migraine showed increased hypothalamic brain activations in the preictal phase [173], as confirmed by a longitudinal study of one woman with daily fMRIs for one month [174]. Akerman et al. [415] hypothesized altered response to stressors and homeostatic changes in migraineurs, causing activation and sensitization of the trigeminal nociceptive pathways through altered control by the PAG-RVM and descending projections from the hypothalamic nuclei. The initiation of preictal symptoms may be due to increased expression of OFF-cells, which will simultaneously increase the nociceptive threshold. This fits with the preictal hypoalgesia that we found in the present study. Thus, both thalamocortical hypoexcitability and increased OFF-cell discharge by hypothalamic activation are plausible, although unspecific, explanations of the low preictal pain scores. Indeed, because the hypothalamus and thalamocortical loops are highly connected, alterations at both sites may be the result of shared pathophysiology.

A mechanism termed “thalamocortical dysrhythmia” has been proposed to be operant in several neurological and psychiatric conditions, such as tinnitus, Parkinson’s disease, depression, schizophrenia, and neuropathic pain [416-418], as well as migraine [151]. Either top-down or

bottom-up reduced excitatory drive or excess inhibition may engender thalamic membrane hyperpolarization and trigger low-frequency theta rhythmicity in thalamocortical loops. Normal cortical high-frequency activity release GABA onto neighboring cells, a process termed lateral inhibition [419]. Thalamic low-frequency activation of cortical inhibitory neurons may reduce lateral inhibition and result in adjacent increases in high-frequency gamma-oscillations [416]. This simultaneous activation of theta and gamma oscillations is termed the "edge effect" [420]. Coppola et al. [151] found increased early (subcortical [421]) visual evoked gamma band oscillations in migraineurs, and decreased habituation of late (cortical) oscillations, consistent with thalamic disconnection combined with a decreased cortical lateral inhibition [422]. Results from previous EEG and pain studies fit with a concept that combines thalamocortical hypoexcitability with sensory hypersensitivity [327]. The present results did not confirm a general preictal hypersensitivity but suggest that early preictal hyposensitivity may replace interictal hypersensitivity, which again may evolve into ictal hypersensitivity. A cortical "normalization" in the preictal phase has been proposed based on visual and auditory evoked potentials studies [423, 424], but contrary to our findings, the normalization of evoked potentials in those studies extended throughout the attack. Inter- and intra-individual differences seem to contribute to variability between published findings, making these fluctuating mechanisms challenging to study. Also, as demonstrated with different evoked potential measures [425], the present findings do not support an altered brain sensitivity state across modalities in migraine.

The transition to the ictal phase

It is important to realize that a neural system may respond normally to a few single stimuli, depending on neural "preactivation" or "tone", while the response to repeated stimuli in some situations may be increased, like in long-term potentiation [426], epileptic kindling [427], or in temporal summation of pain in neuropathic-pain patients [428]. Hence, concepts like hyperresponsivity, hypoexcitability, and hyperexcitability depend on context. Several studies support fluctuations of cortical responsivity over time in migraineurs. A review of the response properties of the cortex in migraineurs suggested that the cortex was hyperresponsive between attacks [429]. The lack of habituation and decreased (or regular) initial response to evoked responses supported a reduced preactivation level of sensory cortices. Early high-frequency oscillations, considered to reflect thalamocortical activation, were reduced before (but not during) the migraine attack [430]. Accordingly, habituation deficits present interictally may normalize during attacks. The preactivation level of sensory cortices may be reduced because of inefficient thalamocortical drive in the period between attacks [429]. However, recent studies have challenged previous findings of interictal habituation deficits by visual evoked potentials [10], like the findings of normal habituation in Paper III. Also, previous findings of normalization of habituation during attacks were reversed in our

findings as we showed normal interictal habituation and deficient ictal habituation. In fact, high-frequency oscillations and somatosensory evoked potentials have demonstrated considerable variation between migraineurs in the interictal phase, showing both increased and decreased responses compared to controls [431]. Furthermore, studies of responses evoked by noxious stimuli have shown reduced habituation both interictally and ictally [324]. Hence, the results vary considerably, and we cannot draw firm conclusions.

Suggestions for further studies

There has been increasing interest in the period preceding the migraine headache. Both triggers and preictal symptoms have been thoroughly mapped, and the number of studies exploring the preictal phase with imaging and neurophysiological techniques is increasing. They have provided evidence of altered central processing of nociception hours to days before a headache, and the preictal alterations in Study II add to these findings. However, to explore the preictal phase in more detail, future studies should increase the temporal and spatial resolution. For instance, headache-diaries with higher temporal resolution should be obtained to be able to characterize shifts in the migraine cycle in more detail. For instance, it is possible that hyperexcitability occurs as early as the late preictal phase.

The cyclical changes in pain perception and adaptation processes should be compared to other modalities of experimental pain. Different experimental pain modalities may represent different pathways [432]. A multimodal approach, in combination with specific protocols able to differentiate the sources responsible for pain modulation and maintenance of homeostasis, might shed light on the pathophysiological mechanisms. For instance, assessment of neuronal oscillations and synchrony in response to noxious stimuli [404, 433-438], with optimized source modeling [86], possibly in combination with alteration by rTMS of deeper brain regions, such as the insula and ACC [439]. Further, specialized paradigms testing different aspects of the endogenous analgesia mechanisms that inhibit pain, like the spatial filtering by conditioned pain modulation or the temporal filtering by offset analgesia [440-443], may prove useful. To better monitor shifts in arousal, one could measure cognitive event-

related potentials and cognitive state changes with appropriate tests of speed, accuracy, inhibition, etc. (e.g., Conners Continuous Performance Test [444]).

Migraine and the hypothalamus

Migraineurs seem to cope less well with changes in circadian rhythm, and most attacks begin in the morning [445-449]. Sleep disturbances are associated with headache [450-452], and lack of sleep may provoke headache [453]. Insufficient sleep in migraineurs may induce hyperalgesia and precipitate attacks [217]. Thus, migraine may be linked to circadian mechanisms, but the effect of sleep depression on experimental pain in migraineurs should be further examined. Migraine may also be associated with disturbances in autonomic control [454-457]. The hypothalamus with its connections to brainstem areas likely participates in maintenance of homeostasis by autonomic control, pain modulation, and sleep regulation [458]. Sleep alterations, autonomic control, and pain may be a promising combination of measures in future examinations of the preictal phase.

Non-invasive deeper brain stimulation

Stimulation of DPINS and the medial parietal operculum (medial part of S2) may be of interest [459], and there are substantial implications of this region in pain processing [460]. For instance, it is the major site of projection of the spinothalamic pathway [461], it shows consistent activation in response to nociceptive stimuli [69, 462, 463], stimulation can lead to pain sensations, or trigger painful epileptic seizures [73, 464, 465], and lesions alter normal nociceptive processing and can lead to neuropathic pain [466, 467]. Indeed, electrical stimulation of DPINS with implanted electrodes was effective in altering HPT [468]. In addition to DPINS, subtle alterations in ACC may be present in migraineurs [469, 470]. Therefore, both insula and ACC are interesting sites in future rTMS studies of migraine.

The figure-of-eight rTMS coil we used has been widely used recent years. It produces an electric field that is more focal than circular type coils, exhibits a good depth-focality tradeoff [471], and may be more reliable [472]. However, the stimulus depth is limited to about 2 cm [473, 474], but may be increased to 3-4 cm if a sufficiently strong intensity is used [475]. Other coils have been designed to achieve more in-depth stimulation, e.g., double-cone coils [287] or H-coils [476]. Interestingly, the double-cone coil may achieve a stimulation depth of 4-5 cm [477, 478], enough to reach DPINS which lies about 5 cm deep [287]. Also, the double-cone coil has been applied in studies of ACC-stimulation [479, 480]. Thus, the double-cone coil may be a promising device for rTMS to insula and ACC.

Methodology

As always, optimized and transparent methodology is a prerequisite for credible and reproducible results. Migraineurs are a heterogeneous group, and valid comparisons between studies depend on thorough presentations of characteristics and methods of inclusion/exclusion of participants. Contextual factors profoundly influence the processing and subjective sensation of pain. Thus, blinding of diagnosis and the various conditions under study, like migraine phase, both during data recording and analysis, is essential for confirmatory studies and real progress in the study of migraine pathophysiology.

Conclusion

The present findings, summarized in Table 4, suggest that migraineurs have altered cortical mechanisms responsible for maintaining homeostasis. Subtle interictal hyperexcitability may alternate with short-lasting preictal hypoexcitability, evidenced by decreased pain ratings and supplementary analyses (Appendix), and ictal hyperexcitability. It can be hypothesized that fluctuations in descending pain-controlling OFF-cell activity, possibly mediated by cortical hypoexcitability or increased intracortical inhibition, may characterize the instability around the preictal phase. The autonomic symptoms associated with the preictal and ictal phase, in addition to recent image findings, may suggest thalamocortical alterations (dysrhythmia) by hypothalamic modulation as a generator of the observed preictal hypoalgesia.

Although habituation of LEPs was equal between groups, both cold allodynia and lack of the expected effect by rTMS to S2 may indicate subtle altered brain responsivity in the interictal phase by the same mechanisms as discussed in the preictal phase. We found interictal alterations at both hand and face, indicating a central process like central sensitization, as opposed to the regional cephalic worsening during the ictal phase. The complex interplay between cortical and subcortical structures, especially in the preictal phase, needs further investigation.

We also emphasize that effect sizes for significant measures were too small to have probable clinical relevance for individual patients. Therefore, the search for a reliable, valid neurophysiological marker in migraine must continue, and future longitudinal studies aimed at exploring the migraine phases should apply measures that are even more specific, in a very systematic manner, in order to map regions of interest for future efforts. Such measures are partly available (e.g., conditioned pain modulation-

methods for quantification of supraspinal pain inhibition by OFF-cells, genetic methods, laser-evoked cortical synchronizations and desynchronizations for cortico-cortical and intracortical oscillatory networks, somatosensory evoked high-frequency oscillations and spindle quantification for thalamocortical function, and navigated TMS with depth-stimulation coils), and partly they will depend on future technological developments. It is still an overwhelming challenge to dissect the multiple cortical and subcortical neural networks that interact to produce the overall pain experience and alter homeostasis in migraine patients.

Appendix

A pilot study of rTMS effects on thermal pain thresholds and suprathreshold tonic pain scores in the preictal phase (Supplementary data to Paper I)

Aim and methods

Our aim was to compare the effects of navigated rTMS to S2 on thermal pain thresholds and suprathreshold tonic pain scores between migraineurs in the preictal and interictal phase. We provided a general description of subjects and methods in Paper I.

Supplementary Table 1 *Demographic and clinical data.*

	Interictal (*n* = 26)	Preictal (*n* = 7)
Age mean (SD) [range], years	27 (8) [20-51]	28 (9) [22-42]
BMI mean (SD), kg/m^2	24 (6)	25 (4)
Women, *n* (%)	23 (88)	6 (86)
Days since 1st day of last menstrual period, mean (SD)	17 (18)	10 (5)
MwoA, MA+MwoA, MA, *n* (%)	15 (58), 4 (15), 7 (27)	4 (57), 1 (14), 2 (29)
Years with headache mean (SD) [range]	13 (8) [2-34]	18 (12) [4-35]
Migraine days/month mean (SD) [range], 0-4[a]	1.5 (0.6) [1-3]	1.9 (0.7) [1-3]
Migraine intensity mean (SD) [range], 1-4[b]	2.6 (0.6) [1-3]	2.4 (0.5) [2-3]
Headache duration mean (SD) [range], hours[c]	11 (14) [1-60]	18 (25) [2-72]

[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), MA migraine with aura (in 100 % of attacks). NA not applicable.

Background data for the preictal subgroup were similar to the interictal subgroup (Supplementary Table 1). We discarded one preictal recording of suprathreshold pain scores due to technical difficulties. Therefore, we analyzed seven preictal thermal threshold recordings and six preictal pain score recordings.

Results

In explorative analyses comparing the thresholds and pain scores between baseline, sham, and rTMS, none of the interactions between thresholds or pain scores, and rTMS were significant, interpreted as no difference in the effect of rTMS between interictal and preictal groups. Trigeminal baseline-measurements of CPT differed between groups, as shown by higher preictal CPT (i.e., trigeminal preictal cold suballodynia, estimated difference = 5.9 [0.6, 11.1] °C, $p = 0.029$). Trigeminal baseline pain scores after 30 seconds were lower in the preictal group (i.e., trigeminal preictal heat hypoalgesia, estimated difference = 2.1 [0.2, 4.1] NRS-score, $p = 0.035$). CDT, WDT, and HPT did not differ between groups. The temperature needed to elicit initial pain ratings of NRS = 6 was not different between interictal and preictal migraineurs ($p > 0.55$).

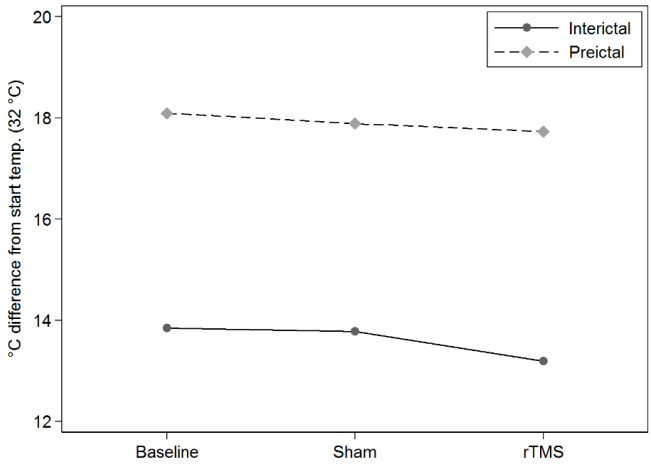
Supplementary Table 2 *Mean (SD) Cold and heat pain thresholds, and pain scores.*

	Cold pain thresholds		Heat pain thresholds		Pain scores	
	Interictal	Preictal	Interictal	Preictal	Interictal	Preictal
Hand						
Baseline	15.91 (6.26)	18.38 (5.79)	9.48 (3.27)	9.87 (3.76)	5.85 (2.64)	4.00 (2.90)
Sham	15.24 (6.97)	18.23 (5.01)	10.37 (3.27)	9.84 (3.50)	4.60 (2.55)	3.00 (3.29)
Real rTMS	15.03 (6.66)	18.51 (5.91)	10.02 (3.18)	10.63 (2.90)	4.87 (2.74)	2.83 (3.54)
Forehead						
Baseline	11.77 (6.86)	17.81 (6.50)	9.03 (3.69)	10.63 (4.05)	4.94 (2.07)	2.33 (2.94)
Sham	12.37 (7.45)	17.55 (6.44)	9.48 (3.80)	10.91 (4.36)	4.13 (2.63)	1.92 (2.58)
Real rTMS	11.35 (7.10)	16.95 (6.98)	8.72 (3.44)	10.31 (3.34)	4.48 (2.41)	2.42 (3.32)

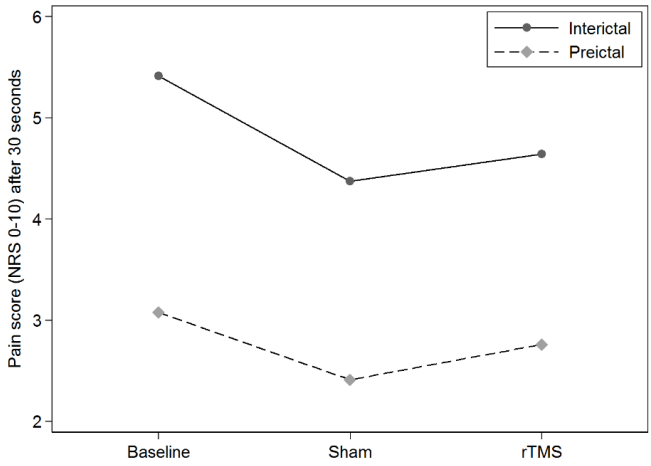
Discussion

The effect of rTMS did not differ between migraineurs in the preictal and interictal phase. However, forehead CPT was higher in the preictal phase, suggesting suballodynia, in contrast to previous findings [4]. Pain scores after 30 seconds were lower compared with interictal recordings, indicating reduced pain perception preictally. Since rTMS to S2 cortex had no effect, subcortical hypoexcitability (e.g., at a

hypothalamic or PAG-RVM-level) can be hypothesized as a possible explanation. However, a cortical origin cannot be excluded as only S2 was targeted in the present study. The preictal group comprised only seven subjects and paired within-subject comparisons were unavailable. The findings should accordingly be considered preliminary, and these data were for these reasons not included in Paper I.



Supplementary Figure 1 *Estimates of CPT by group and stimulation for both sites combined.*



Supplementary Figure 2 *Estimates of Pain scores by group and stimulation for both sites combined.*

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Paper I

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Non-invasive cortical modulation of experimental pain in migraine

M. Uglem [a,*], P.M. Omland [a], M. Engstrøm [a,b], G.B. Gravdahl [b], M. Linde [a,b], K. Hagen [a,b], T. Sand [a,b]

[a] Norwegian University of Science and Technology, Department of Neuroscience, Trondheim, Norway
[b] St. Olavs Hospital, Department of Neurology and Clinical Neurophysiology, Trondheim, Norway

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HIGHLIGHTS

- The effects of rTMS to S2 on thermal pain thresholds differed between migraineurs and controls.
- The analgesic effects of rTMS to S2 were of low magnitude.
- The results may suggest a hypofunction of the descending pain-modulating system in migraineurs.

ABSTRACT

Objective: To test the hypothesis that secondary somatosensory cortex (S2) is involved in the migraine pathogenesis, by exploring the effect of navigated repetitive transcranial magnetic stimulation (rTMS) to S2 on thermal perception and pain.
Methods: In this blinded sham-controlled case-control study of 26 interictal migraineurs and 31 controls, we measured thermal detection and pain thresholds on the hand and forehead, and pain ratings to heat stimulation on the forearm and temple, after real and sham 10 Hz rTMS.
Results: rTMS increased cold and heat pain thresholds in controls as compared to interictal migraineurs ($p < 0.026$). rTMS decreased forehead and arm pain ratings ($p < 0.005$) and increased hand cool detection thresholds ($p < 0.005$) in both interictal migraineurs and controls.
Conclusions: The effects of rTMS to S2 on thermal pain measures differed significantly between migraine and control subjects, although the effects were generally low in magnitude and not present in pain ratings. However, the lack of cold and heat pain threshold increase in migraineurs may reflect a hypofunction of inhibitory pain modulation mechanisms.
Significance: The expected rTMS-induced cold and heat hypoalgesia was not found among migraineurs, possibly a reflection of reduced intracortical inhibition.

1. Introduction

The migraine pathophysiology is partly unknown, but it is generally accepted that dysfunction of central nervous system (CNS) structures is involved, causing unstable CNS-excitability. This dysfunction could cause migraine attacks by increasing the susceptibility for activation and sensitization of the trigeminovascular pain pathway (Vecchia and Pietrobon, 2012; Noseda and Burstein, 2013).

Many structures are involved in modulation of nociceptive signals before the conscious recognition of pain. The primary somatosensory cortex (S1) and secondary somatosensory cortex (S2) are likely involved in the sensory-discriminative aspects of pain, while the insula and the anterior cingulate cortex are involved in motivational-affective aspects of pain (Xie et al., 2009). S2 may also be involved in modulation of pain (Kuroda et al., 2001; Gojyo et al., 2002). The activation of S2 by experimental pain may be decreased in interictal migraineurs compared to controls (Schwedt et al., 2015).

Repetitive transcranial magnetic stimulation (rTMS) can non-invasively modulate cortical excitability in humans. Although these effects are far from homogeneous, it seems that

Abbreviations: CDT, cool detection threshold; CPT, cold pain threshold; DLPFC, dorsolateral prefrontal cortex; fMRI, functional magnetic resonance imaging; HPT, heat pain threshold; M1, primary motor cortex; MEP, motor evoked potentials; rANOVA, repeated measures analysis of variance; RMT, resting motor threshold; rTMS, repetitive transcranial magnetic stimulation; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; WDT, warm detection threshold.

* Corresponding author at: Department of Neuroscience, Medisinsk teknisk forskningssenter, N-7491 Trondheim, Norway. Tel.: +47 470 25 765; fax: +47 73 59 87 95.
E-mail address: martin.uglem@ntnu.no (M. Uglem).

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low-frequency rTMS (⩽1 Hz) decreases and high-frequency rTMS (⩾5 Hz) increases excitability (Lefaucheur et al., 2014).

Interictal migraineurs may have lower thermal pain thresholds compared to controls (Schwedt et al., 2011; Engstrom et al., 2013). The pain thresholds may further decrease right before and during migraine attack (Burstein et al., 2000; Sand et al., 2008). More than half of migraineurs experience allodynia closely before a migraine attack in questionnaire-based studies (Mathew et al., 2004; Lipton et al., 2008), and allodynia has been associated with increased responses in the thalamus, insula and S2 (Lorenz and Casey, 2005). Although S2 is partly involved in pain processing, and most likely its modulation, it has not been widely used as a target for pain modulation by rTMS (Mylius et al., 2012). However, in one study navigated high-frequency rTMS to S2 increased heat pain thresholds in healthy subjects, and resulted in a more pronounced and longer lasting alteration compared to stimulation to M1, S1 and dorsolateral prefrontal cortex (DLPFC) (Valmunen et al., 2009).

To test the hypothesis that S2-excitability is involved in the migraine pathogenesis, it would be of interest to compare the effects of navigated rTMS to S2 on thermal pain thresholds and suprathreshold pain ratings in interictal migraineurs compared to healthy controls, since alteration of nociception may be a more clinically relevant measure than measures of motor cortex excitability. In addition, we studied the effect of rTMS on thermal detection thresholds (as secondary variables) to look for unspecific effects on the sensory system. As far as we know, this is the first study exploring the effect of navigated rTMS to S2 in migraineurs (Moisset et al., 2015).

2. Methods

In this blinded sham-controlled case-control study, we measured thermal perception and pain thresholds and ratings from prolonged noxious heat stimulation before and after high-frequency rTMS to S2. Migraineurs kept a headache diary for four weeks before and after the examinations in order to determine the relationship between migraine attacks and the examination day. Measurements were classified as interictal when they were performed more than one day before attack onset or more than one day after the attack ended.

2.1. Subjects

Forty-three migraineurs and 34 healthy controls participated in the study. Participants were students and employees recruited through an Intranet advertisement within our university. Migraineurs were included by neurologists according to the ICHD-II criteria for migraine with and without aura (Headache Classification Subcommittee of the International Headache Society, 2004). Included subjects should have between two and six migraine attacks per month and no more than ten days with migraine per month. Symptomatic, but not prophylactic, migraine medications were allowed.

Exclusion criteria were coexisting frequent episodic (1–14 days/month for healthy controls and 7–14 days/month for migraineurs) or chronic (>15 days/month) tension-type headache, 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.

Nine subjects were excluded (six with migraine); five due to technical difficulties with the magnetic coil, two due to sleepiness (one interictal and one postictal migraineur), one due to technical difficulties with the thermal test equipment, and one because we were unable to determine resting motor threshold (RMT). Migraineurs who were classified by the headache diary to be either ictal ($n = 3$), preictal ($n = 7$) or postictal ($n = 1$) were excluded prior to statistical analysis. Twenty-six interictal migraineurs and 31 healthy controls were finally included (Table 1). 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 $ 80 to cover expenses.

2.2. Procedure

Magnetic resonance imaging scans (3-T Siemens Trio MRI scanner, T1 weighted 3D sequence) were acquired before the neurophysiological procedure. All participants were examined at the same time of day and were told to avoid exercise, smoking and caffeine-containing beverages the morning before examination to reduce the influence of factors that may affect the effect of rTMS (Ridding and Ziemann, 2010). The examination consisted of determination of RMT, baseline thermal tests before rTMS and new thermal tests after real and sham rTMS. Both real and sham rTMS were applied on all participants in a randomized order with 45 min between the first and second rTMS session.

2.2.1. Navigated transcranial magnetic stimulation

The stimulation setup consisted of a figure-of-eight shaped coil with biphasic pulse of 280 μs duration (MCF-B65 Butterfly Coil, MagVenture A/S, Farum, Denmark), a magnetic stimulator (MagPro

Table 1
Demographic and clinical data.

	Healthy controls ($n = 31$)	Interictal migraineurs ($n = 26$)
Age mean (SD) [range], years	30 (10) [19–56]	27 (8) [20–51]
BMI mean (SD), kg/m^2	24 (6)	24 (6)
Women, *n* (%)	26 (84)	23 (88)
Days since 1st day of last menstrual period, mean (SD)	18 (13)	17 (18)
MwoA, MA + MwoA, MA, *n* (%)	NA	15 (58), 4 (15), 7 (27)
Years with headache mean (SD) [range]	NA	13 (8) [2–34]
Migraine days/month mean (SD) [range], 0–4[a]	NA	1.5 (0.6) [1–3]
Migraine intensity mean (SD) [range], 1–4[b]	NA	2.6 (0.6) [1–3]
Headache duration mean (SD) [range], hours[c]	NA	11 (14) [1–60]

[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). MA = migraine with aura (in 100% of attacks). NA = not applicable.

X100 with MagOption, Medtronic A/S, Skovlunde, Denmark) and a tracking unit with navigation software (eXimia NBS Navigation System 2.2, Nexstim Ltd., Helsinki, Finland). MEPs were recorded with 9 × 6 mm pre-gelled disposable surface electrodes (Alpine Biomed ApS, Skovlunde, Denmark) attached over the belly of the right hand abductor pollicis brevis muscle and connected to a Viking Select system (Nicolet Biomedical Inc., Madison, WI USA) with filters set for a band-pass between 2 Hz and 10 kHz.

The participants sat in a comfortable reclining chair. Individual magnetic resonance imaging scan files were loaded into the navigation software for live navigation. With the coil's current direction oriented perpendicular to the central sulcus, we started stimulating the area in the left motor cortex most likely representing movement of the right hand. To identify the site with the largest and most reproducible peak-to-peak MEP, we started with a coarse mapping to narrow down the area before a more careful mapping. The sites with the largest MEP were stimulated again to check for reproducibility and consistency. The coil was then rotated horizontally (within the coil-plane) to find the optimal orientation on the chosen site.

A relative frequency method based on the Rossini criterion was used to determine the RMT (Rossini et al., 1994). RMT was defined as the lowest stimulator intensity needed to elicit MEPs with peak-to-peak amplitudes of at least 50 μV in five out of ten consecutive trials. A suprathreshold stimulus was reduced in steps of five units, until less than five out of ten recorded MEPs were large enough. The stimulus intensity was then increased by four units and decreased by one unit until less than five out of ten trials were positive. RMT was defined as the last trial where at least five out of ten MEPs were above 50 μV.

2.2.2. Navigated high-frequency rTMS

rTMS was delivered to the S2 area (above the posterior subcentral sulcus in Sylvian fissure) with anteroposterior current direction. The contralateral side to the side with most frequent migraine pain was stimulated. If it was equally often on either side, or both, the choice of side was randomized. Nine-hundred stimuli were given with intensity 90% of RMT. The stimuli were separated in 18 trains of 50 stimulations at 10 Hz with 10 s between-trains intervals. An rTMS-session lasted for 4 min and 20 s. Sham-stimulation was conducted with the coil tilted 90 degrees pointing downwards with anteroposterior current direction (Lisanby et al., 2001). One wing of the coil touched the subjects head at the same site as the active stimulation. The subjects were not informed that the procedure included sham stimulation and could not see the position of the coil.

2.2.3. Thermal sensory testing

The thermal tests were measured with SOMEDIC SenseLab equipment (Somedic Sales AB, Stockholm, Sweden). The hand (thenar eminence overlying the abductor pollicis brevis muscle) and forehead (frontal region above the eyebrows aligned with the inner canthus) were stimulated with a hand-held rectangular 25 × 50 mm Peltier element thermode (Somedic Sales AB, Stockholm, Sweden). The target start temperature was 32 °C and the actual start temperature was recorded by the system and was stable = 32.2 °C. The stimulation range was 5–50 °C with 1 °C/s slope.

Innocuously cool and warm detection thresholds (CDT and WDT respectively), and cold and heat pain thresholds (CPT and HPT respectively) were measured on the hand and forehead contralateral to the side of S2 stimulation, using the method of limits. The subjects were lying supine on a bench with a stop-button in the hand opposite to the stimulated side. Each threshold was measured four times consecutively with randomized 4–6 s inter-stimuli intervals. The order was always the same: CDT, CPT, WDT and HPT; first on the hand, then the same order on the forehead. The participants were told to press the stop-button as soon as they felt an increase or decrease in temperature when testing cool and warm detections. When measuring pain thresholds, they were instructed to press the button immediately when the stimulus was perceived as "pain". An introductory round was carried out at the beginning of the day, consisting of two measurements of each threshold on the hand.

Suprathreshold heat pain scores were measured on the right forearm and temple. The individually determined tonic temperature that was scored as 6 on a numerical rating scale (NRS), ranging from 0 = "no pain" to 10 = "unbearable pain", was set as the test stimuli. We used the same equipment and thermode as when testing thresholds, controlled by the software Exposure30 by SOMEDIC. The start-temperature target was set at 32 °C, and the slope was 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 the highest score was less than six and decreased the temperature if the highest score 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 a 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 test procedure consisted of one continuous stimulation per site with 30 s duration. Degree of pain was reported continuously. The pain at 30 s was stored for analysis.

2.3. Data analysis

Thresholds were defined as difference from the measured start-temperature (dCDT = start − CDT, dWDT = WDT − start, dCPT = start − CPT and dHPT = HPT − start). Outlier detection software was applied, removing single responses with magnitude more than three times or less than one third of the mean of the three associated responses.

STATA (StataCorp LP, version 13.1, College Station, TX USA) was used to run separate multilevel linear mixed-effects models (Rabe-Hesketh and Skrondal, 2012) for each response variable (dCPT, dHPT, dCDT, dWDT and pain rating). The analyses of thermal thresholds included subject-specific random intercepts and random slopes for Stimulation (Baseline, Sham and rTMS) and Site (Forehead and Hand) with an unstructured variance–covariance matrix. dCPT and dHPT were fitted with one residual variance, while dCDT and dWDT achieved better fit with an independent variance by Site. The fit was tested with −2Log Likelihood, Akaike's information criterion and Bayesian information criterion. Parsimonious models were preferred, hence, Bayesian information criterion was the decisive criterion. Analysis of pain rating was specified with random intercepts for subject, but no random slope to prevent "overfitting" the model (due to only one measurement within each combination of categorical groups). The fit improved with individual residual variances grouped by Site. The maximum likelihood estimator can be significantly biased if the number of degrees of freedom is sufficiently small (Harville, 1977). Therefore, restricted maximum likelihood estimation was used to estimate variance components. Normal distribution of the random coefficients and residuals were visually checked with histograms. dCDT and dWDT were transformed to the power of −0.5 to improve normality.

The fixed factors were determined by the research hypotheses. The main goal was to test the effect of rTMS between groups, i.e. the interaction Stimulation × Group. Stimulation was dummy

coded with base at active rTMS in order to compare the effect of rTMS to both Sham and Baseline. The interactions Group × Site and Stimulation × Site were also included. Group and Site were dummy coded with base at Migraine and Forehead, respectively. The three-way interaction Group × Stimulation × Site was not included in the model because it was not a part of the research hypothesis, complicated the interpretation of the Stimulation × Group interaction and did not improve the fit. A significant Stimulation × Group interaction would reflect different responsivity to rTMS (as compared to Sham or Baseline depending on the current sub-interaction) between controls and migraineurs. Post hoc analyses of significant interactions were applied to inspect the simple effects of rTMS at each level of Group and Site.

Individual temperatures used for suprathreshold tonic heat stimulation were compared between groups with independent Student's *t*-tests. Results were considered significant at a level of $p < 0.05$. Šidák's method of adjustment were applied to post hoc analyses to account for multiple comparisons.

3. Results

3.1. Thermal pain thresholds

Mean pain thresholds between controls and migraineurs are displayed in Table 2. Significant Stimulation × Group interactions were found for dCPT and dHPT (Fig. 1). These results suggest that rTMS affect thermal pain thresholds differently in interictal migraine as compared to controls. Both pain thresholds increased more in controls than migraineurs after rTMS compared to sham ($p < 0.015$). The increase in dHPT was also significant as compared to baseline ($p = 0.026$), and a trend was observed for the increase in dCPT as compared to baseline ($p = 0.088$). Post hoc inspection of the simple effects of Stimulation across the levels of Group and Site show an increase in dCPT in controls after rTMS compared to sham for both sites ($p = 0.002$). Similarly, hand dHPT increases in controls after rTMS compared to baseline ($p < 0.001$). The effect on dHPT in controls is not significant compared to sham, but comparison of rTMS versus sham shows a significant decrease in forehead dHPT in migraineurs ($p < 0.013$).

3.2. Detection thresholds

rTMS did not affect detection thresholds differently between groups, but hand dCDT increased compared to forehead dCDT after rTMS compared to both sham and baseline ($p < 0.005$, Fig. 2). Post hoc analyses show that the effect was significant in both groups and due to an increase in hand dCDT ($p < 0.029$) without significant effect on forehead dCDT.

Table 2
Mean (SD) thermal pain thresholds before (baseline), after sham rTMS and after real rTMS in controls ($n = 31$) and interictal migraineurs ($n = 26$). Thresholds are expressed in mean °C difference from start temperature (32 °C).

	Cold pain thresholds (dCPT)		Heat pain thresholds (dHPT)	
	Controls	Migraine	Controls	Migraine
Forehead				
Baseline	14.66 (7.91)	11.77 (6.86)	9.12 (3.55)	9.03 (3.69)
Sham	14.25 (7.93)	12.37 (7.45)	9.67 (3.86)	9.48 (3.80)
rTMS	16.01 (8.21)	11.35 (7.10)	9.68 (3.91)	8.72 (3.44)
Hand				
Baseline	16.77 (6.31)	15.91 (6.26)	9.66 (3.18)	9.48 (3.27)
Sham	15.81 (6.81)	15.24 (6.97)	10.96 (3.38)	10.37 (3.27)
rTMS	16.95 (6.29)	15.03 (6.66)	11.12 (3.35)	10.02 (3.18)

3.3. Suprathreshold heat pain ratings

Pain ratings from both sites decreased in both groups after rTMS compared to baseline ($p < 0.005$, Fig. 3). The effect was not significant compared to sham ($p > 0.261$). The temperature needed to elicit initial pain ratings of NRS = 6 was lower in migraineurs than controls for temple (44.5 and 46.0; mean difference = −1.6 [−2.9, −0.2] °C, $p = 0.025$) and forearm (46.1 and 44.4; mean difference = −1.5 [−2.6, −0.3] °C, $p < 0.001$).

4. Discussion

The main finding in this blinded sham-controlled study was that rTMS-modulation of thermal pain thresholds differed in interictal migraineurs compared to control subjects. dCPT and dHPT increased significantly after high-frequency navigated rTMS to S2 in controls as compared to migraineurs. Another main observation was the generally low effect-magnitudes of rTMS to S2 on experimental pain thresholds and ratings; in general below 1.5 °C as compared either to sham or to baseline. Hence, the clinical value of the presently applied rTMS-protocol is uncertain although navigated low-frequency rTMS to S2 reduced pain in patients with severe visceral pain (Fregni et al., 2011) and neuropathic orofacial pain (Lindholm et al., 2015).

The mechanisms of induced analgesic effects by rTMS to S2 are not clear. In fact, the underlying mechanisms of sustained excitability modulation by rTMS are not fully understood, but probably involve long term potentiation-like mechanisms (Pell et al., 2011). The analgesic effects by rTMS probably involve many brain structures and depend on pain modulatory systems, see (Moisset et al., 2015) for a recent review. The vast majority of studies that have explored the analgesic effects of rTMS in humans stimulated M1 or DLPFC, and concluded that the stimulation effects mainly depend on mechanisms other than a direct inhibition of the spinal transmission of nociceptive signals (Moisset et al., 2015). However, the analgesic effects of S2-stimulation may differ from M1 or DLPFC-stimulation since S2 is primarily involved in the sensory-discriminative aspects of pain (Xie et al., 2009). The available evidence is not sufficient to draw accurate conclusions, but the lack of analgesic effects in migraineurs may represent a change in cortical pain-processing, possibly an altered activation of pain inhibitory mechanisms, resulting in a hypofunction of the pain-modulating system. Such a hypofunction may contribute to hyper-responsivity to external stimuli (Coppola et al., 2007), thus rendering the cortex more sensitive to external stimuli, and less capable of adapting to homeostatic changes. This may predispose to a migraine attack (Coppola et al., 2015).

Resting-state functional magnetic resonance imaging (fMRI) studies have identified several functional connectivity abnormalities in migraineurs (Colombo et al., 2015; Schwedt et al., 2015). For instance, the periaqueductal gray, an important part of the brainstem pain-inhibiting circuitry, has shown significantly greater functional connectivity with several brain regions measured by fMRI, including S2, in interictal migraineurs compared to controls (Mainero et al., 2011). Since the subject-specific cortical excitability and connectivity before stimulation influences the effect of rTMS (Lefaucheur et al., 2014; Nettekoven et al., 2015), the altered functional connectivity in migraineurs may partly explain the lack of rTMS-effect. Indeed, several studies with priming of the excitability before rTMS of M1 have shown altered effects in migraineurs compared to controls (Brighina et al., 2005,2010,2011; Cosentino et al., 2014).

Cerebellum is also involved in pain perception (Moulton et al., 2010; Baumann et al., 2015), and may have altered functional connectivity in migraineurs (Chen et al., 2015). A study of brain

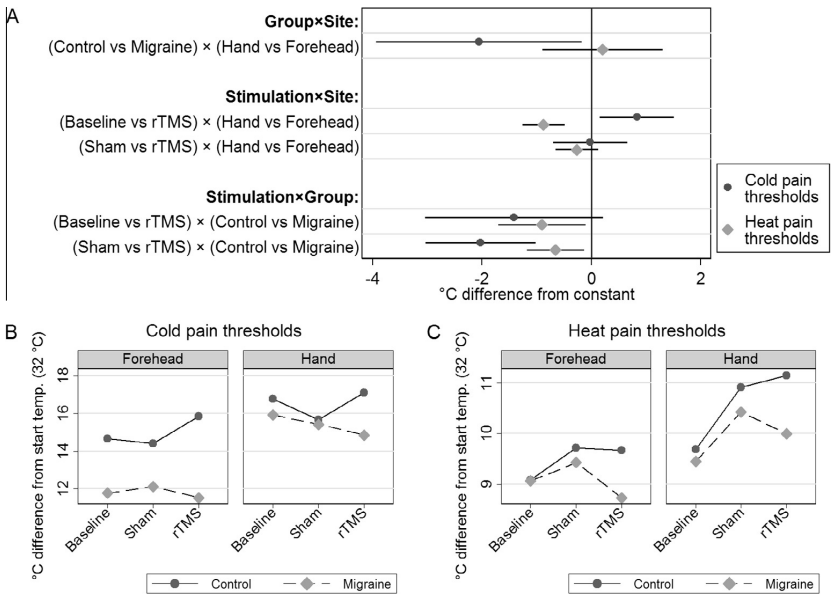

Fig. 1. The effect of navigated rTMS to S2 on pain thresholds. (A) Predicted pain threshold coefficients with 95% confidence intervals from interactions of Group, Stimulation and Site. Simple effects are not shown. Intervals that do not contain zero are significant at $p < 0.05$. The constant is set to zero and represents forehead thresholds from migraine after rTMS. The cold pain threshold constant was estimated to 11.5 and the heat pain threshold constant to 8.7 °C difference from start temperature (32 °C). The Stimulation × Group interactions test the main hypothesis comparing the effect of navigated rTMS to S2 on pain thresholds in interictal migraineurs compared to healthy controls. A negative coefficient in these interactions means an increase in pain thresholds in controls as compared to migraine, after rTMS as compared to baseline or sham. (B) Adjusted predictions of cold pain thresholds by group, stimulation and site. Thresholds increased after rTMS compared to sham in controls in both sites. (C) Adjusted predictions of heat pain thresholds by group, stimulation and site. Hand thresholds increased after rTMS compared to baseline in controls. Forehead thresholds decreased after rTMS compared to sham in migraineurs.

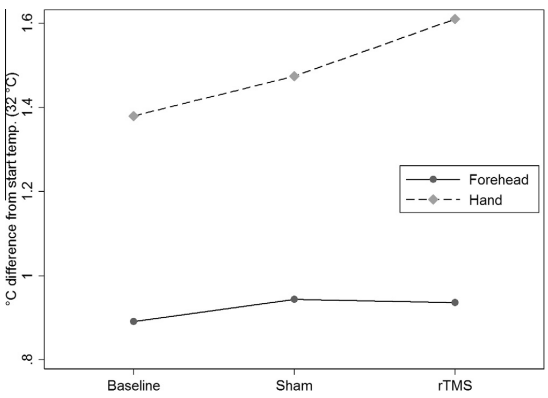

Fig. 2. Adjusted predictions of cool detection thresholds by site and stimulation for both groups combined. Group differences are not shown due to no significant group differences. Hand cool detection thresholds increased after rTMS compared to both sham and baseline.

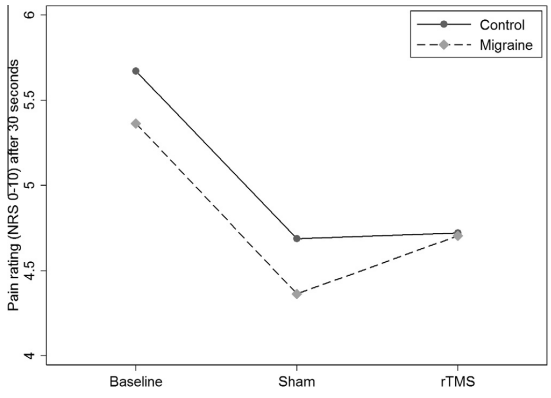

Fig. 3. Adjusted predictions of pain ratings after 30 s of suprathreshold heat stimulation by group and stimulation for both sites combined. Site differences are not shown due to no significant site differences. Pain ratings decreased after rTMS compared to baseline in both groups.

network connectivity during induced migraine attacks found decreased resting-state functional connectivity, measured by fMRI, between cerebellum and the "default mode network" hours before migraine pain was experienced, which may suggest lack of cerebellar nociceptive modulation (Amin et al., 2016). Hence, cerebellum seems to be an interesting target for rTMS or transcranial direct current stimulation in future migraine studies (Bocci et al., 2015).

We demonstrated decreased pain ratings after 30 s of tonic heat stimulation in both migraineurs and controls after rTMS compared to baseline. Two minutes of tonic heat stimulation has been shown to produce a typical pain rating response curve in most subjects (Potvin et al., 2008; Redmond et al., 2008; Tousignant-Laflamme et al., 2008; Potvin et al., 2012; Suzan et al., 2015). Initially, pain ratings increase followed by temporary decrease and gradual increase during the second minute. The second increase possibly reflects temporal summation of pain (Tousignant-Laflamme et al., 2008), the psychophysical correlate of wind-up (Eide, 2000). A-delta fibers are probably the source to the initial rise and fall

because of rapid firing before gradually wearing out and a transition to a predominantly C fiber response occurs (Tillman et al., 1995; Treede, 1995; Tousignant-Laflamme et al., 2008). Therefore, the difference in pain ratings after 30 s may predominantly represent differences in C fiber activity. Furthermore, rTMS did not alter maximal pain ratings (data not reported), generally occurring earlier than 30 s in the "A-delta time window", suggesting that the effect of rTMS to S2 on pain ratings during tonic heat stimulation mainly decrease perception of pain mediated by C fibers. However, it is uncertain if rTMS actually contributed to this decreased perception, as the effect of rTMS on suprathreshold pain was similar to the effect of sham stimulation. Migraineurs reached a pain rating of six at a lower temperature compared to controls, indicating interictal hyperalgesia and peripheral or central sensitization (IASP, 2012).

The increased pain thresholds after rTMS seen in our control group is comparable to the findings of Valmunen et al. (2009) who found increased facial HPT and, in a sub-analysis of male participants, increased CPT. Previous studies in healthy subjects have demonstrated analgesic effects by stimulation of different sites and with different frequencies. Stimulation of left DLPFC with 10 Hz rTMS increased thermal pain thresholds (Borckardt et al., 2007) and lowered pain ratings (Martin et al., 2013) compared to sham rTMS. Low-frequency 1 Hz rTMS of the right DLPFC increased cold pressor tolerance during stimulation (Graff-Guerrero et al., 2005), and 10 Hz rTMS of both right M1 and DLPFC increased thermal pain thresholds (Nahmias et al., 2009). Studies examining the effect of rTMS on experimental pain in migraineurs are sparse. One study found a reduced laser-evoked potentials amplitude over vertex in migraineurs compared to controls after 5 Hz rTMS of M1, but the rTMS-effect did not differ from sham stimulation and the pain rating was unaffected (de Tommaso et al., 2010). Based on these studies and our results, rTMS seems to increase pain thresholds and decrease pain ratings in healthy subjects, but only affect pain ratings in migraineurs. However, the analgesic effects are small and variable.

We found an increase in hand dCDT after rTMS in both controls and migraineurs. Imaging studies have shown S2-activation by several different innocuous stimuli, including innocuous temperatures. The activity enhances with increasing temperature and show a marked increase in response when reaching painful ranges (Peyron et al., 2000). Intracranial recordings of laser-evoked potentials demonstrated enhanced responses within S2 with increasing stimulus intensity, but the responses did not increase further when stimuli passed the pain threshold. However, within the insula, response magnitudes continued to increase for stimulus-intensities above pain threshold (Frot et al., 2007). These findings support that stimulation of S2 primarily can be expected to alter detection thresholds, as demonstrated by increased dCDT after rTMS compared to both baseline and sham in the present study. However, Valmunen et al. (2009) demonstrated no effects of navigated rTMS to S2 on CDT. Stimulation of other sites has also shown different results on the effect on CDT. Stimulation of M1 with 1, 5 and 20 Hz rTMS has previously been shown to increase CDT in healthy subjects (Summers et al., 2004; Oliviero et al., 2005), although 10 Hz rTMS of M1 decreased CDT in one study (Nahmias et al., 2009), and both CDT and WDT in another study (Lefaucheur et al., 2008).

4.1. Strengths and limitations

We used a standard figure-of-eight coil that can activate the motor cortex at a distance of two centimeters (Zangen et al., 2005). This may not be sufficient in order to reach the area most active in pain modulation. Garcia-Larrea (2012) argues that the suprasylvian posterior insula and *medial* operculum constitutes the "primary cortex for pain". The S2 region corresponds to the *lateral* operculum, also labelled OP1 and OP4 (Eickhoff et al., 2006), i.e. it is not a part of the "primary cortex for pain". However, OP1 has been shown to be activated by both innocuous and noxious stimuli, while pain stimuli induced intense activation in both OP1 and OP4 (Mazzola et al., 2012). It is therefore reasonable to assume that the figure-of-eight coil reached areas active in detection of temperature and pain changes. However, a coil that can activate deeper areas might have a greater potential for modulation (Ciampi de Andrade et al., 2012) as suggested from work with other stimulation modalities; e.g. electrical stimulation of insula increased HPT in a small group of epilepsy patients with implanted electrodes (Denis et al., 2015).

We applied a real-time frameless stereotaxic system to ensure a precise localization of S2 and to improve the reliability of coil placement throughout the session. Only a few of the experimental studies referenced in this paper applied navigation (Valmunen et al., 2009; Fregni et al., 2011; Hasan et al., 2014; Lindholm et al., 2015). Navigation is superior to non-navigated procedures because it takes into account the large inter-subject variability in brain morphology (Lefaucheur, 2010). Lack of navigation may be an important source for the lack of consistency of findings in previous studies.

We emphasized the importance of generating a truly inactive sham. Tilting the coil 90° induces lower voltage-differences in the brain compared to 45° (Lisanby et al., 2001), and touching the scalp with the lateral edge of one wing is better than with the front edge (Loo et al., 2000). Hence, we are confident that the sham stimulation we applied did not produce a partially active sham. The coil contact area, the sound and the "hammering" sensation from the coil were virtually equal. However, some subjects experienced activation of the temporalis muscle during active stimulation, which were absent during sham. We randomized the order of presentation of stimulation, sham vs active first, to control for order effects. The order of tested sites during thermal testing was kept constant, hand before forehead and forearm before temple. The main aim of the study was to compare thresholds before and after rTMS, hence constant order of testing was preferred. However, interpreting results between sites becomes more complex.

5. Conclusions

The reduced analgesic effect of rTMS on thermal pain thresholds in migraineurs may represent a slightly reduced activation of inhibitory pain modulation mechanisms in migraineurs, a hypofunction that renders the cortex more sensitive to external stimuli, possibly also contributing to the onset of a migraine attack. Protocols that enable stimulation of more medial regions of S2 and insula may have greater analgesic effect and increase the potential differences of pain modulatory mechanisms between migraineurs and controls.

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

Martin Uglem[1], Petter Moe Omland[1,2], Kristian Bernhard Nilsen[1,3,4], Erling Tronvik[1,2], Lars Jacob Stovner[1,2], Knut Hagen[1,2], Mattias Linde[1,2] and Trond Sand[1,2]

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 the 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 the 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 changes within the migraine cycle is important to gain a more complete understanding of the pathogenic mechanisms behind the migraine attack.

Keywords

Headache, migraine cycle, preictal, premonitory, allodynia, hyperalgesia

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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 60% of migraineurs report cutaneous allodynia during headache (the 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, such as 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 precede and outlast the ictal phase.

[1]Department of Neuroscience, Faculty of Medicine, NTNU, Norwegian University of Science and Technology, Trondheim, Norway
[2]Department of Neurology and Clinical Neurophysiology, St. Olavs Hospital, Trondheim, Norway
[3]Department of Work Psychology and Physiology, National Institute of Occupational Health, Oslo, Norway
[4]Department of Neurology, Section for Clinical Neurophysiology, Oslo University Hospital, Ullevål, Norway

Corresponding author:
Martin Uglem, Department of Neuroscience, Medisinsk teknisk forskningssenter, N-7491 Trondheim, Norway.
Email: martin.uglem@ntnu.no

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, that is, 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 had to 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 the 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 greater than or equal to seven days 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. (50)

Investigators were blinded to diagnosis on the subjects' first visit, and to migraine phase on the subsequent visits. Co-workers undertook inclusion,

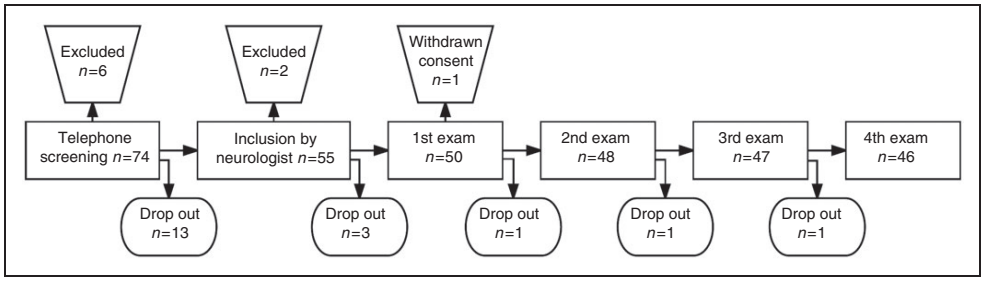

Figure 1. Flow chart for the migraineurs in the study. The number of subjects who dropped out due to personal reasons is shown at the bottom.

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 first 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.

coordination and follow-up of participants, and participating subjects were specifically told not to reveal to 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 the equivalent of $125 and $30 respectively, to cover expenses.

Procedure

All sessions on one subject were at 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 25×50 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 four to six 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 six on a verbal numerical rating scale (NRS), ranging from 0 = no pain to 10 = unbearable pain, was set as the test stimulus (52). We used the same

equipment and thermode as when testing thresholds, controlled by the Exposure30 software 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 it if NRS was more than six. At least three stimuli were applied on both sites with a minimum of a one-minute inter-stimulus interval on the same site. The temperature perceived as the 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. Subjects 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 stimulus temperature. The same individually determined temperatures were used for the next three examination days.

Data analysis

Thresholds were defined as the 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 magnitudes of 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. The first three models compared migraineurs' within-subject change by migraine phase and site. In addition, to explore adaptation and

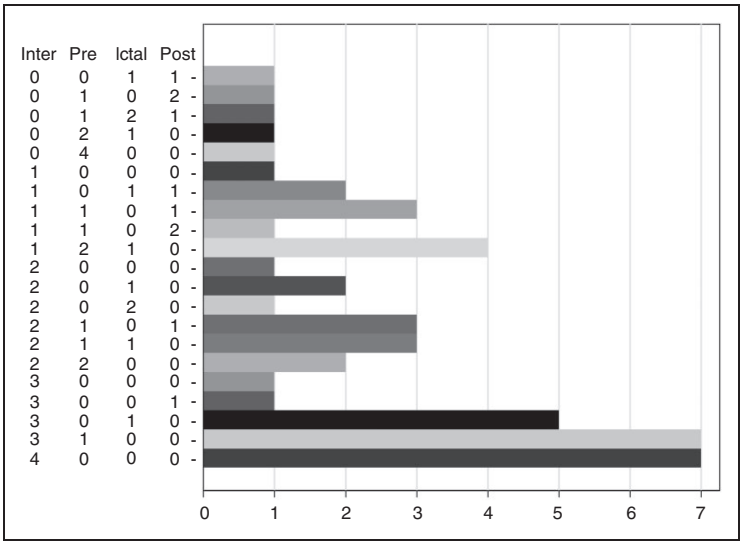

Figure 2. Bar graph showing the distribution of phase combinations among migraineurs. The labels on the y-axis represents the number of exams in each phase (interictal, preictal, ictal and postictal, respectively). Hence, for example, 2,1,0,1 means two interictal, one preictal, zero ictal and one postictal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

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

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

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

To test if there was a linear relationship between pain thresholds and scores and time to the 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

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, see Supplementary Appendix for further description.

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

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 within each group.
*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, see Supplementary Appendix for further description.

"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 at 80%. As we estimated having 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 at 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 showed that ictal forehead dCPT was 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 were 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, either for the interictal group or 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

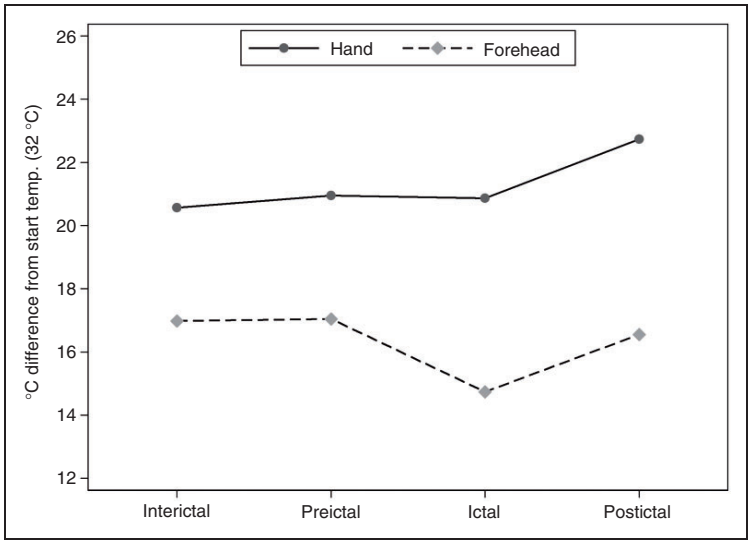

Figure 3. Cold pain thresholds. Graphical display of estimated margins from the main multilevel model comparing the effects of phase and site on cold pain thresholds. Ictal forehead thresholds were significantly decreased compared to interictal forehead thresholds. The decrease was within the normal range, thus interpreted as trigeminal suballodynia in the ictal phase.

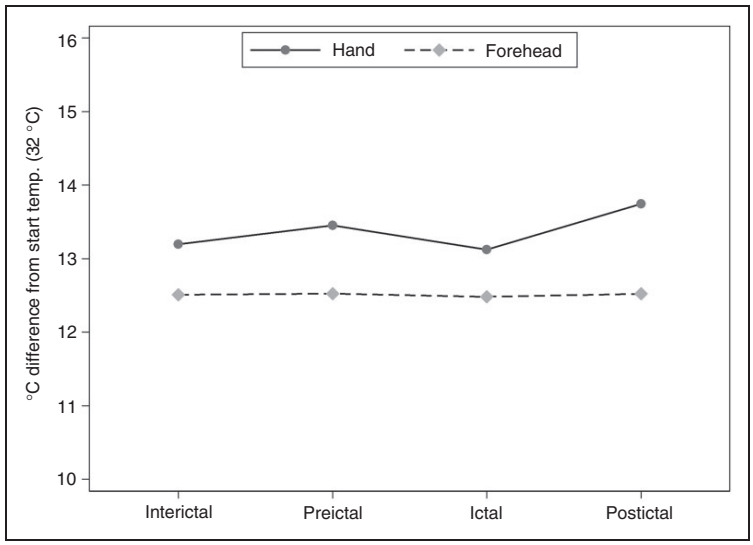

Figure 4. Heat pain thresholds. Graphical display of estimated margins from the main multilevel model comparing the effects of phase and site on heat pain thresholds. There were no significant differences between phases.

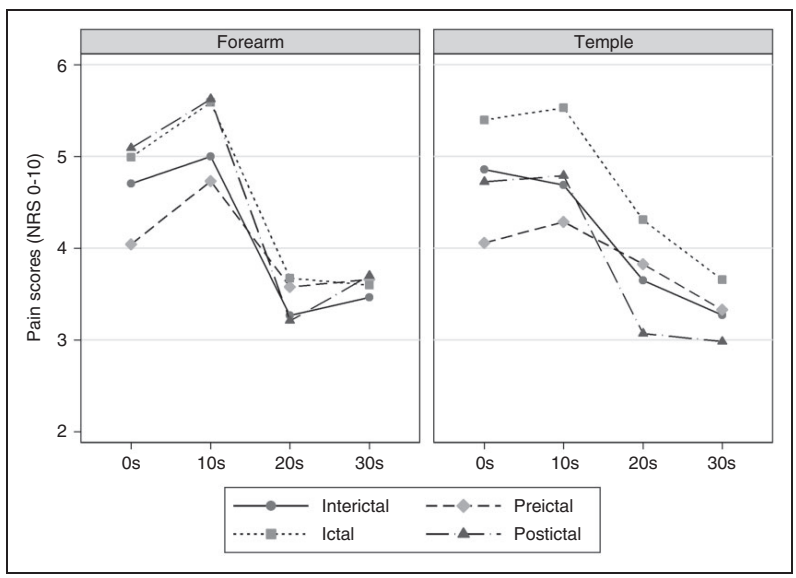

Figure 5. Pain scores during continuous suprathreshold heat pain stimulation. Graphical display of estimated margins from the main multilevel model comparing the effects of phase, site and time on pain scores. The x-axis represents the four time-points at which pain scores were recorded during 30 seconds of tonic heat. The overall pain scores at the temple were increased ictally compared to interictally, interpreted as trigeminal hyperalgesia in the ictal phase. At time point 0s, preictal pain scores were decreased at both sites compared to interictal pain scores, interpreted as initial preictal hypoalgesia. There were interictal decreases in pain scores from 0s to 20s and 0s to 30s at both sites, interpreted as interictal adaptation of pain scores. The preictal pain scores decreased significantly less, interpreted as preictal lack of adaptation.

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-day 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 the initial cortical processes responsible for the prodromal symptoms are 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 would 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 the proximity 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 the ictal compared to the 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 headache, as expected. However, this general pattern was interrupted for a limited time-window preceding headache, interpreted as preictal hypoalgesia. These results suggest that significant central events affect processing of pain on the day before headache.

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

The observed temporal profile of pain scores during continuous suprathreshold heat pain stimulation in the present study is at variance with some (52,56,57), but

not all previous studies (58–62). Migraineurs demonstrated lower initial pain and significantly less adaptation in the preictal compared to the interictal phase. A-delta fibers may be important for the initial rise and fall in pain scores observed in the first 15 seconds of the continuous suprathreshold heat pain stimulation (60,63,64). Our observed lower pain scores could have reflected a blunted preictal A-delta nociceptive response, but since a central mechanism is most probable, we interpret this finding as a blunted preictal saliency perception.

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

Studies comparing experimental pain in migraineurs and controls have shown variable results; either hypersensitivity (1–13) or no differences (1,5,9,12,36,67,68,71–76), but never hyposensitivity. Some subgroups may be more hypersensitive than others; for instance, migraineurs with non-sleep related migraine attacks had lower CPT and HPT than controls (77), while less slow wave sleep was associated with higher pressure pain thresholds (1). Disease severity may also be of importance, as headache history duration may modulate CPT (36), while chronic migraineurs (>15 days/month) were more sensitive to pain compared to episodic migraineurs in one study (66), but not in another (9). Headache frequency correlated with temporal summation of electrical and mechanical stimulation (12) and pressure thresholds (67), although there are contradictory findings (4). Thermal pain thresholds did not correlate with headache frequency, allodynia symptom severity, anxiety scores or depression scores (10). Migraine is divided into subgroups of subjects with and without aura, but these groups did not differ in the present study and do not seem to differ systematically by pain thresholds in previous studies (5,36). Thus, since a multitude of factors may influence sensitivity in individual patients, this heterogeneity may explain why results regarding pain thresholds and other sensitivity measures vary between studies.

Strengths and limitations

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

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

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

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

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

The final dCPT-model was defined as a three-level model with measurements nested in days nested in subjects. A random slope for site with an unstructured covariance structure was added at the second

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

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

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)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Phase and site were dummy-coded with Interictal and Forehead as baseline, respectively. Thus, the constant represents interictal forehead pain thresholds. Pain thresholds are presented as difference from start temperature (32°C). Heat pain thresholds were squared before estimation.

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

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Phase, site and time were dummy-coded with Interictal, temple and 0s as baseline, respectively. Thus, the constant represents interictal temple pain scores at 0 seconds.

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Paper III

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RESEARCH ARTICLE Open Access

Habituation of laser-evoked potentials by migraine phase: a blinded longitudinal study

Martin Uglem[1,4*], Petter Moe Omland[1,2], Marit Stjern[1,2], Gøril Bruvik Gravdahl[3] and Trond Sand[1,2]

Abstract

Background: Migraineurs seem to have cyclic variations in cortical excitability in several neurophysiological modalities. Laser-evoked potentials (LEP) are of particular interest in migraine because LEP specifically targets pain pathways, and studies have reported different LEP-changes both between and during headaches. Our primary aim was to explore potential cyclic variations in LEP amplitude and habituation in more detail with a blinded longitudinal study design.

Methods: We compared N1 and N2P2 amplitudes and habituation between two blocks of laser stimulations to the dorsal hand, obtained from 49 migraineurs with four sessions each. We used migraine diaries to categorize sessions as interictal (> one day from previous and to next attack), preictal (< one day before the attack), ictal or postictal (< one day after the attack). Also, we compared 29 interictal recordings from the first session to 30 controls.

Results: N1 and N2P2 amplitudes and habituation did not differ between preictal, interictal and postictal phase sessions, except for a post hoc contrast that showed deficient ictal habituation of N1. Habituation is present and similar in migraineurs in the interictal phase and controls.

Conclusions: Hand-evoked LEP amplitudes and habituation were mainly invariable between migraine phases, but this matter needs further study. Because hand-evoked LEP-habituation was similar in migraineurs and controls, the present findings contradict several previous LEP studies. Pain-evoked cerebral responses are normal and show normal habituation in migraine.

Keywords: Headache, Migraine cycle, Pain, Pathophysiology, Preictal, Ictal, Premonitory, Laser evoked potential, Habituation, LEP

Background

Migraine is a cyclic disorder as evidenced by subjective symptoms and imaging and neurophysiological studies [1–7]. Therefore, it is preferable to investigate migraine physiology repeatedly during the different phases, i.e., between, before, during and after attacks (interictal, preictal, ictal and postictal phase, respectively) [8, 9].

Laser-evoked potentials (LEP) are well suited to study the cortical response to noxious input since brief laser pulses mainly evoke cortical responses with a latency corresponding to the conduction velocity of Aδ fibers [10–14]. Aδ fiber activation yields a middle-latency component over the contralateral temporal lobe (N1) and a late biphasic vertex response (N2P2). The operculo-insular cortex and possibly the primary somatosensory cortex largely contributes to N1 [11, 15, 16], while the anterior cingulate cortex contributes to N2P2 [11]. Hence, LEP may reflect both pain-specific activation of the primary sensory cortex and cognitive and inhibitory "top-down control" aspects of pain physiology in migraine.

LEPs in migraineurs have mainly been studied by an Italian collaboration [17–24]. The results are not entirely coherent, but deficient N2P2-habituation has been observed in the interictal phase [17–19, 21, 22], a deficit that seems to persist during attacks [21]. Deficient LEP-

* Correspondence: martin.uglem@ntnu.no; https://www.ntnu.edu/inb
[1]Department of Neuromedicine and Movement Science, NTNU, Norwegian University of Science and Technology, Trondheim, Norway
[4]NTNU, Faculty of Medicine and Health Sciences, P.B. 8905, N-7491 Trondheim, Norway
Full list of author information is available at the end of the article

habituation has also been observed in painful radiculopathy [25], fibromyalgia [26] and cardiac syndrome X [27]. The apparently reduced LEP-habituation in migraineurs do not differ systematically between stimulation sites [17, 18, 20–22], and whether the N1 or N2P2-potential are best suited to demonstrate an alteration is not clear [17, 20]. Accordingly, these results for LEP N2P2 should be independently confirmed [28]. N1-habituation should also be studied further in migraine as only two studies have recorded this early LEP-component [17, 20].

LEPs or other pain evoked potentials have, as far as we know, not been investigated previously in the preictal or postictal phases. In the interictal phase, lack of habituation of the nociceptive blink reflex and pain scores to repeated noxious stimuli has been shown [29, 30], with a tendency towards normalization during the migraine attack [30, 31]. However, several studies have measured evoked responses to repeated non-nociceptive stimuli in migraineurs. The results are conflicting regarding visual evoked potentials (VEP) as some studies show reduced habituation in migraineurs between attacks while others do not [9, 32, 33]. Most migraine-studies of evoked potentials habituation have focused on the interictal phase, but some have also investigated cyclic changes. One such study showed normal habituation of the standard blink reflex interictally and decreased habituation in the preictal phase [34]. However, several studies have shown an opposite effect with deficient habituation of VEP, visual evoked magnetic fields, somatosensory evoked potentials, and contingent negative variation between attacks that normalizes right before or during the attack [35–44]. One study has shown increasing loss of habituation of VEP during the interictal interval with a normalization within the migraine attack [45], while other longitudinal studies did not find VEP or brainstem auditory-evoked potential habituation differences related to the migraine cycle [4, 5]. It is accordingly of interest to extend the knowledge about general phase-related neurophysiological changes in migraine to the cortical pain-processing network.

The primary aim of the present blinded longitudinal study was to investigate generalized "third order neuron" pain network excitability in migraineurs by LEP amplitude and habituation during different stages of the migraine cycle. We examined 50 migraineurs four times to investigate intraindividual changes at both the interictal, preictal, ictal and postictal phases. We test the main working hypothesis that LEP amplitude and habituation, and subjective pain scores to laser stimulation, differs between phases. The secondary aims were to confirm previously reported deficient LEP habituation in migraineurs in the interictal phase compared to controls, and to test the effect of aura, headache laterality, years lived with migraine, and subjective pain scores on habituation and habituation-differences between phases.

Methods

We measured LEPs and pain scores once a week for four weeks in migraineurs (mean ± SD: 6.7 ± 1.9 days between sessions) in the second half of 2012. The four sessions in one migraineur were at the same time each day for almost all subjects, but for a few subjects, it was necessary to reschedule one or two sessions. Mean variation between the latest and the earliest of the four sessions were 23 ± 28 min, and the variation was no more than an hour in 41 of 49 subjects. At most, one subject had to postpone two sessions by 3.5 h. The migraineurs completed a headache diary for four weeks before, during and four weeks after the examinations to determine how the examinations were related to the migraine attacks (i.e., interictal, preictal, ictal or postictal). We measured LEPs and pain scores once in headache-free controls. Investigators were blinded to diagnosis on subjects' first visit and migraine phase on the subsequent visits. Co-workers performed the inclusion and follow-up, and participating subjects were specifically told not to reveal their diagnosis to the investigators.

Subjects

Seventy-four migraineurs and 40 controls responded to an advertisement in the local newspaper, on the local hospital's web page [46] and the Intranet within our university [47]. We screened both groups over telephone and migraineurs were evaluated by neurologists per the ICHD-II criteria for migraine with or without aura [48]. Controls could not have a headache more than once a month. If they occasionally had a headache, we asked if they had consulted a physician regarding the headache, if they experienced the headache as painful and if they used abortive medication for their headache. We excluded controls if they confirmed more than one of these three questions. Included migraineurs 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 week 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, substance abuse, ferromagnetic implants and prophylactic allergy treatment.

Fifty migraineurs and 31 controls participated in the study. 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. We excluded one control because we were unable to obtain reliable LEPs as most trials were rejected. Thus, 49 migraineurs completed a total of 190 examinations, and 30 controls completed one examination each. Table 1 shows demographic and clinical data. We report details of exclusions and dropouts in Additional file 1: Table S1.

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

Painful heat stimuli were generated by a pulsed solid-state (Nd:YAP) laser (STIMUL 1340, DEKA M.E.L.A. SRL, Calenzano (FI), Italy) with a wavelength of 1340 nm. The laser stimulator settings were the same as in a previous study at our lab [49]: The pulse duration was 6 ms, a relatively short stimulus duration to maximize the N1-amplitude [50]. We set the laser beam diameter to 8 mm (area ≈ 50 mm^2) with an energy ranging from 2 to 6.5 J (4.0–12.9 J/cm^2). The diameter and durations are comparable to other researchers using the same type of laser [51, 52]. A diode laser aiming beam visualized the stimulation site. We recorded LEPs with a Viking Select system (Nicolet Biomedical Inc., Madison, WI, USA). The recording silver disc electrodes were placed at the Fz, Cz, Pz, T3, T4, A1 and A2 sites of the 10–20 system. The impedance was kept below 5 kΩ. The two most important analysis channels, Cz referred to the nose, and T3 referred to Fz, were preselected as recommended by the international IFCN-guidelines [53]. We used the other channels as back up to account for interindividual variation in field topography and to improve detectability of waves. For control of artifacts, we monitored the electrooculogram from a left infraorbital electrode referred to T4. The onset of stimuli triggered the recording system. The sampling rate was 1000 Hz, the sweep time was 750 ms and the filter setting was 0.2–100 Hz. Rejection level was set to ±225 μV, and total rejection rate after exclusions was 3 %. We applied online averaging [49, 54] since rejection effectively canceled artifacts and eye movements also were included in a separate channel.

Subjects lay comfortably on an examination table with laser safety glasses and acoustic earmuffs to avoid any acoustical interference at the time of stimulation [53, 55]. We delivered laser stimuli to the dorsum of the right hand between the carpal bones, metacarpophalangeal joints and second and fourth metacarpal bone. The laser beam was moved randomly within this area to avoid skin lesions and nociceptor fatigue or sensitization [56]. We measured skin temperature before the test. Because we previously observed that the recommend fixed intensity (equal to twice the mean pin-prick threshold [12]) did not always elicit pain and LEP in every healthy subject [49], we used stimulus intensities based on *intraindividual* thresholds [51, 57, 58]. First, the individual thresholds for pinprick pain were identified, starting at 2 J and increasing with 0.5 J steps [49]. The subject had to differentiate between burning pain and pinprick pain. Subjects scored pinprick pain on a verbal, numerical rating scale (NRS) with range 0 = "no pain" to 10 = "unbearable pain." We measured the threshold twice, and we defined the pinprick threshold as

Table 1 Demographic and clinical data after exclusions

	Migraineurs (n = 49)	Controls (n = 30)
Age	40 ± 10 [19–62]	38 ± 11 [21–59]
BMI	26 ± 3	25 ± 3
Women	*41* (84%)	*25* (83%)
Days since 1st day of last menstruation	17 ± 12	19 ± 10
MwoA, MA + MwoA, MA	*27* (55%), *18* (37%), *4* (8%)	NA
Years with headache	21 ± 9 [1–40]	NA
Migraine days/month[a]	1:*14*, 2:*30*, 3:*5*, 4:*0*	NA
Migraine intensity[b]	1:*2*, 2:*20*, 3:*27*, 4:*0*	NA
Headache duration[c]	16 ± 21 [0.5–72]	NA
Energy level (J) used in LEP test	4.3 ± 0.5	4.4 ± 0.4
Thresholds (J) for pinprick pain	3.7 ± 0.6	3.7 ± 0.7

[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 (hours) of an attack with or without use of symptomatic medication
Data displayed as mean ± SD [range] or n (%). MwoA: migraine without aura. MA + MwoA: some attacks with and some without aura (both diagnoses according to ICHD-3 Beta [88]. MA: migraine with aura (in 100% of attacks). NA: not applicable

the lowest intensity inducing pain in at least one of the two trials at that intensity. A tolerable intensity was 4.5 J (9.0 J/cm^2), in most subjects, corresponding to about two times the pinprick threshold. However, 4.5 J generated too much pain in 22 subjects, and too little pain in five subjects and the energy had to be adjusted up or down (range: 3–5.5 J ≈ 6.0–10.9 J/cm^2). The chosen intensity did generally elicit reliable N2P2 potentials [59]. We recorded two blocks of 21 stimulations with six to ten seconds between each stimulation since six seconds was recommended as the minimum interval to avoid peripheral nociceptor habituation [53]. The between-block interval was also short, between 6 and 10 s, to prevent recovery of central habituation. Subjects kept their eyes open and rated perceived pain verbally (NRS 0–10) after each stimulation to prevent LEP-amplitude decrease by distraction and drowsiness [12, 60, 61]. We stored pain scores for analysis. We used identical energy levels for all sessions within subjects. However, we did not tell participants that the energy was constant.

Data analysis and statistics

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 (a migraine headache during the examination) and postictal (less than one day after the attack ended). We applied this definition in previous studies of pain physiology related to migraine phase [6, 62]. Eleven of the 190 examinations were unclassifiable and excluded from data analysis, mainly because they had migraines both the day before and the day after examination.

We analyzed in LabChart® (Version 7 pro, ADInstruments, Dunedin, New Zealand). A random number identified each LEP session and we randomized the order of the two blocks within each session. Thus, the investigator who analyzed the LEPs was blinded to diagnosis, migraine phase and order of the two blocks. The N1 and the N2P2 components were assessed, N1 at the contralateral temporal electrode (T3) against Fz (best bipolar derivation to show N1 [63]) and N2P2 at Cz against nose [53]. We measured the N1-amplitude from baseline (start of N1) to the N1 peak and the N2P2-amplitude from the most negative to the most positive peak. We had to discard some LEPs due to unrecognizable responses, too much noise/artifacts or latencies far from normal values [53]. The N1-amplitude may have a low signal-to-noise ratio, and it was not detectable in 15% of LEPs in migraineurs and 22% of LEPs in controls. These responses were included in the analysis as interval censored responses [64, 65] by setting the lower bound to zero and the upper bound to the maximal negative noise peak within the N1-time window. The exact N1-amplitude was then unknown, but we presumed that it was between zero and the largest noise peak, and we included the amplitude as an interval, a rough estimate, instead of a point estimate. We discarded recordings with technical errors, 17 of 358 in total in migraineurs. We present the grand average of all recordings by phase (Fig. 1) and by group (Fig. 2).

We analyzed data with STATA version 13.1 (StataCorp LP). We applied separate multilevel models [66] for the response variables N1, N2P2 and pain scores. The first set compared the within-subject change in migraineurs and the second set compared interictal recordings from the first exam and controls. The interaction effects were the main outcomes. For the first set, interactions-of-interest reflect differences between phases. For the second set, interactions-of-interest reflect differences between migraine and healthy subjects. We have included full model specifications in the Additional file 1.

We added explorative post hoc contrasts that tested if the slopes (i.e., habituation) at each phase, and in all phases combined, were different from zero. Also, based on the main results, we performed data-driven explorative post hoc contrasts comparing the first-block amplitudes and habituation slope of N1 between the preictal and ictal phases. We used diagnosis as a fixed factor to compare migraineurs in the interictal phase and controls. Only interictal recordings from the first exam were compared to controls because the investigator was blinded to diagnosis only on the first session, and to avoid possible long-term habituation/sensitization effects in subsequent exams. N1 and N2P2 in both sets were square rooted to improve normality.

We analyzed phase and group differences in pain scores with pain scores from both blocks combined consecutively into one continuous time variable which was interacted with phase or group, respectively. The time variable was centered at its mean and divided by 10. Thus, the regression constant shows the average pain score and the habituation coefficient the change in pain score per ten stimulations. We tested group differences in pain thresholds and laser intensity with independent samples Student's t-test and present results as 95% confidence intervals (CI). Thus we consider intervals not containing zero to be significant at a level of $p < 0.05$. We have back transformed the data and tabulate result-parameters in the original scale. However, results are presented as transformed units in the Additional file 1.

We extended the original models to test the effect of additional variables. We specified four separate models that estimated the phase-differences in habituation for 1) migraineurs with and without aura, 2) sessions differentiated by headache laterality, 3) by years lived with migraine, and 4) by pain scores. Headache laterality was classified by the related attack if the phase was preictal, ictal or postictal. Interictal recordings were classified by

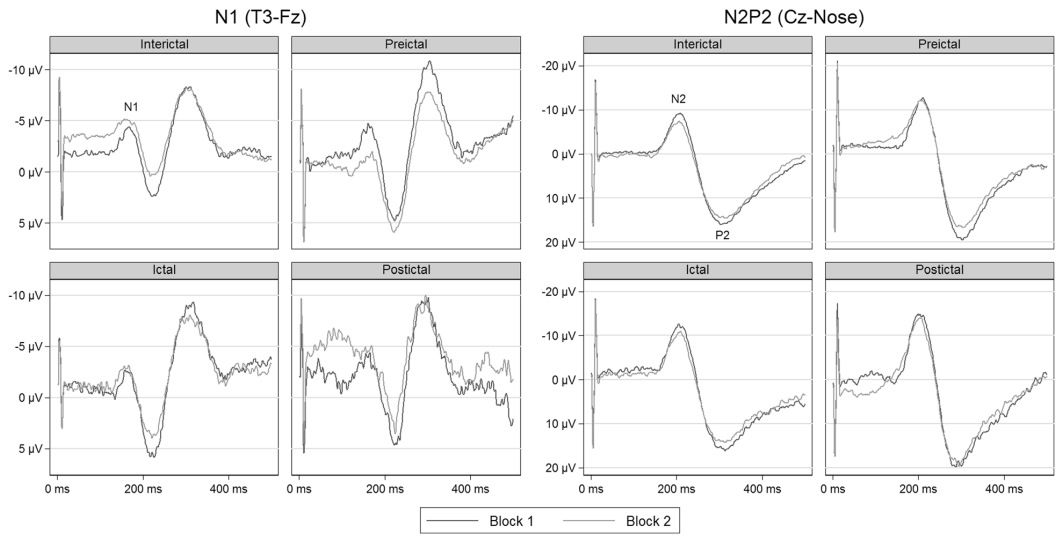

Fig. 1 Grand average of the LEP-traces by phase. Habituation was present in all phases at both LEP-components but ictal and postictal N1, and postictal N2P2. The amplitudes in the figures are smaller than those presented in Table 2 due to slightly different LEP-latencies between participants

the side the subject most commonly experienced headache, either left, right or bilateral. Sixteen interictal recordings had an equal amount of left and right-sided unilateral migraine and were not included in the laterality-analysis. We included age, migraine intensity, and migraine frequency as control variables (not included in the interactions) in the extended model 3 that estimated the effect of years lived with migraine.

We conducted three additional analyses to explore the relationship between habituation and number of days to next attack. We conducted these analyses in two steps, first with interictal phase only and then with both interictal and preictal phases included. We interpret the interaction effects in the latter analyses as the interictal-preictal day-to-day change in habituation towards the next migraine attack. Also, we performed a secondary set of analyses with a three-day limit to test if postictal phase-related LEP-changes last longer than 24 h after the attack.

With 30 controls and 50 migraineurs, the statistical power to detect a low medium-sized effect equal to 0.65 SD [67] based on a two-sample Student's t-test is 80%. As we estimated to have approximately 20 pairs for intraindividual phase-related comparisons, power (based on paired Student's t-tests) to detect a similar medium-sized effect (0.65 SD) was calculated to 83%.

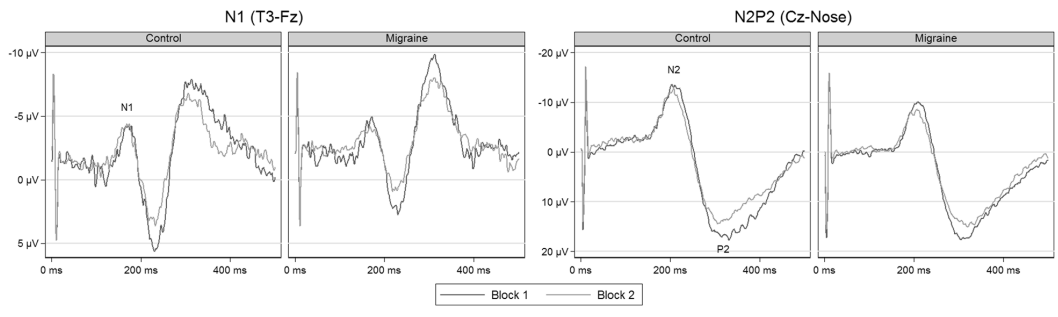

Fig. 2 Grand average of the LEP-traces in first session interictal recordings and controls. N2P2-habituation was present in both groups, but we found no significant N1-habituation. The amplitudes in the figures are smaller than those presented in Table 4 due to slightly different LEP-latencies between participants

Results

Analyses by phase

Table 2 shows means and standard deviations of N1 and N2P2-amplitudes, and pain scores by phase.

N1-habituation was significant in the interictal phase as shown by the negative coefficient of block (Fig. 3 and Table 3). The degree of habituation was not different between the interictal phase and the preictal, ictal and postictal phases respectively (interaction effects in Table 3). However, post hoc contrasts showed significant habituation in the preictal phase (95% CI [−2.71, −0.33] μV/block), but not in the ictal and postictal phases (95% CI [−1.29, 0.57] and [−3.44, 0.00] μV/block, respectively). The habituation in all phases combined was significant (95% CI [−1.40, −0.38] μV/block). The contrast of the difference in habituation between the preictal and ictal phase was not significant (95% CI [−0.54, 2.86] μV/block). Neither the first-block amplitudes nor the combined first and second-block amplitudes differed between phases, but the post hoc contrast that compared first-block amplitudes between the preictal and ictal phases showed a tendency towards lower first-block amplitudes in the ictal phase (95% CI [−3.20, 0.04] μV).

The N2P2-amplitude change from the first to the second block was significant in the interictal phase, and none of the interactions were significant (Fig. 3 and Table 3), interpreted as interictal habituation with no differences between phases. Post-hoc contrasts showed significant habituation in both the preictal and ictal phases (95% CI [−6.66, −1.58] and [−7.38, −0.77] μV/block, respectively), but not in the postictal phase (95% CI [−7.01, 1.22] μV/block). The habituation in all phases combined was significant (95% CI [−4.90, −2.55] μV/block). N2P2-amplitude sizes did not differ between phases.

Pain scores increased linearly in the interictal phase (95% CI [0.11, 0.33] NRS-change/10 stimuli, Table 3). The linear increase, i.e., sensitization of pain scores, was not different between phases. Mean pain scores did not differ between phases.

We present complete results from the secondary analyses in the Additional file 1. N1 and N2P2 first-block amplitudes and habituation did not differ between migraineurs with and without aura as none of the three-way or two-way interactions were significant. Amplitudes and habituation did not differ between a left and right-sided migraine. Subjects with a bilateral migraine had reduced N2P2-habituation (more positive slope) in the postictal phase compared to the interictal phase (95% CI [0.07, 25.3] μV/block), and the same tendency was present in the ictal phase (95% CI [−0.01, 19.8] μV/block). The more years lived with migraine; the less was the N2P2-habituation in the preictal phase compared to the interictal phase (95% CI [0.11, 0.82] μV/block/year adjusted for age). No other interaction effects were significant.

Both N1 and N2P2 interictal first-block amplitudes correlated with pain scores (95% CI [0.08, 0.93] and [1.74, 3.65] μV/unit pain score for N1 and N2P2, respectively). The interactions between phase and pain score were not significant, that is, the correlations were not different between phases. Habituation of N1 and N2P2-amplitudes did not correlate with a change in pain scores from the first to the second block.

The analyses that explored the relationship between habituation and number of days to next attack showed no significant interactions. Thus, there was no interictal day-to-day linear change in habituation towards the next migraine attack. Changing the definition of the postictal phase from a one-day limit to a three-day limit did not alter the interpretation of LEP-habituation. Habituation of pain scores did not change by changing the definitions of the phases, although the mean pain score was significantly increased in the postictal compared to the interictal phase (95% CI [0.11, 1.28] unit pain score).

Analyses by diagnosis

Table 4 shows means and standard deviations of N1 and N2P2-amplitudes, and pain scores by group.

Controls did not show habituation of the N1 amplitude, and habituation did not differ between migraineurs and controls (Fig. 4 and Table 5). Post-hoc contrasts showed no habituation in the groups combined (95% CI [−1.32, 0.30] μV) and no habituation in the migraine group (95% CI [−1.77, 0.49] μV). Neither the first-block

Table 2 N1 and N2P2-amplitudes, and pain scores by phase and block

			N1 (μV)		N2P2 (μV)		Pain scores	
	N	*n*	Block 1	Block 2	Block 1	Block 2	Block 1	Block 2
Interictal	44	99	6.6 (3.5)	5.9 (2.6)	40.2 (16.6)	35.2 (13.8)	4.2 (1.9)	4.3 (2.0)
Preictal	26	36	7.9 (5.0)	6.3 (3.6)	42.3 (13.4)	37.3 (11.2)	4.1 (1.9)	4.4 (2.0)
Ictal	19	21	5.7 (2.2)	5.0 (2.2)	39.6 (12.8)	34.6 (10.1)	4.4 (1.7)	4.7 (1.9)
Postictal	13	15	6.8 (3.0)	4.9 (3.7)	45.7 (13.7)	43.2 (12.5)	5.4 (2.6)	5.7 (2.7)

Mean (SD) N1 and N2P2-amplitudes, and pain scores. The means were calculated in two steps; first, phase-specific means for each subject (most subjects had two or more measurements classified within the same phase), before phase-specific means in all subjects combined. Because some N1-amplitudes were interval censored, i.e., defined only by a minimum and maximum with the actual value somewhere in between, the interval midpoints were used as approximate estimates to calculate the means. *N:* number of subjects with at least one recording at the respective phase. *n*: total number of recordings at the respective phase

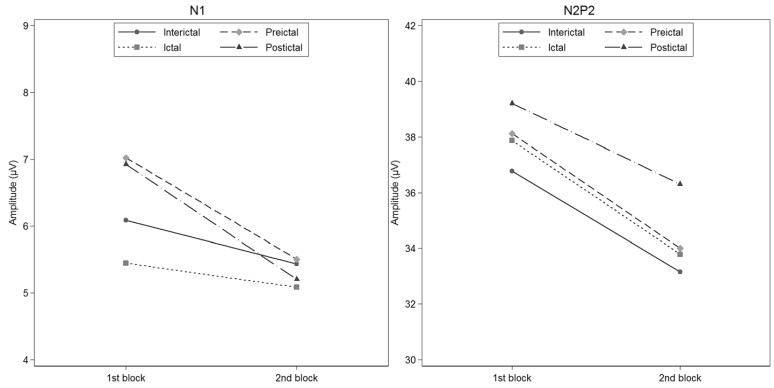

Fig. 3 Estimates of N1 (left) and N2P2-amplitude (right) habituation by phase

amplitudes nor the combined first and second-block amplitudes differed between migraineurs and controls.

The N2P2-amplitude decreased from the first to the second block in controls, and the decrease was not different between controls and migraineurs (Fig. 4 and Table 5). Post-hoc contrasts confirmed a significant habituation in migraineurs (95% CI [−8.07, −2.48] μV). Overall amplitudes were not different between groups (95% CI [−9.87, 5.11] μV).

The linear change in pain scores was not significantly different from zero in controls (95% CI [−0.06, 0.27] NRS-change/10 stimuli) and did not differ between migraineurs and controls (95% CI [−0.09, 0.35] NRS-change/10 stimuli, Table 5). Pain thresholds and stimulation intensities were not different between groups (Student's t-test 95% CI [−0.37, 0.34] and [−0.04, 0.44], respectively).

Discussion

As far as we know, this is the first study to measure cyclic changes of LEP N1-habituation in migraine. Our results show habituation of both N1 and N2P2 amplitudes in all phases combined. In line with the overall responses, both interictal and preictal N1 and N2P2 habituated. Habituation of N2P2 was present in the ictal phase as well. The deficient ictal habituation of N1 was only present in the post hoc contrasts, not in the main analysis, and the number of ictal recordings was relatively small (n = 21). Thus, we interpret the finding of

Table 3 Estimated magnitudes and habituation of N1, N2P2 and pain scores by phase

	N1 (μV)		N2P2 (μV)		Pain scores	
	Coef.	95% CI	Coef.	95% CI	Coef.	95% CI
Main effects						
Preictal	0.939	[−0.476, 2.353]	1.342	[−1.993, 4.677]	−0.01	[−0.36, 0.34]
Ictal	−0.641	[−1.753, 0.470]	1.088	[−2.862, 5.039]	0.23	[−0.28, 0.74]
Postictal	0.839	[−0.487, 2.166]	2.426	[−2.419, 7.272]	0.37	[−0.35, 1.10]
Habituation	−0.653*	[−1.315, −0.001]	−3.623***	[−5.147, −2.098]	0.21***	[0.09, 0.33]
Interaction effects						
Preictal × Habituation	−0.868	[−2.283, 0.547]	−0.497	[−3.445, 2.451]	0.07	[−0.10, 0.24]
Ictal × Habituation	0.293	[−0.746, 1.331]	−0.455	[−4.086, 3.176]	0.06	[−0.18, 0.31]
Postictal × Habituation	−1.070	[−2.890, 0.749]	0.726	[−3.657, 5.108]	0.12	[−0.17, 0.41]
Constant	6.088	[5.186, 6.989]	36.783	[33.002, 40.565]	4.11	[3.56, 4.67]

The constant represents interictal first-block or mean pain score responses, the first three main effects are first-block amplitude or pain score differences from the interictal phase and the fourth "Habituation" main effect is the difference between first and second block, or the linear change of pain scores, in the interictal phase. The interaction effects represent habituation differences between the interictal phase and the preictal, ictal and postictal phases, respectively. Thus, the significant coefficients are interpreted as decreased second-block N1 and N2P2-amplitudes, and linear increase in pain scores, in the interictal phase, i.e. interictal N1 and N2P2 habituation and subjective pain sensitization. Lack of significant interaction effects are interpreted as no habituation differences between the interictal phase and the other phases. Random effects estimates are shown in Supplementary Table 2. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 4 N1 and N2P2-amplitudes, and pain scores in migraineurs in the interictal phase and controls

		N1 (μV)		N2P2 (μV)		Pain scores	
	N	Block 1	Block 2	Block 1	Block 2	Block 1	Block 2
Migraineur	29	7.0 (4.0)	6.1 (3.1)	38.7 (17.5)	33.5 (14.5)	4.2 (2.0)	4.1 (2.3)
Control	30	8.5 (8.6)	7.8 (7.5)	41.1 (16.9)	35.2 (15.3)	3.6 (1.6)	3.5 (1.6)

Mean (SD) N1 and N2P2-amplitudes, and pain scores. The migraine group consists of interictal recordings from the first session. The means and SD of N1-amplitudes are calculated with the interval midpoints of interval censored responses. *N:* number of subjects with a recording of at least one block

deficient ictal N1 habituation with caution, and we believe that it needs to be replicated.

It has been suggested that lack of habituation and normal or slightly decreased first-block amplitudes are functional properties of migraine between attacks [33]. These properties seem to normalize during the attack, at least for non-noxious evoked potentials [9]. The reduced habituation may be due to thalamocortical "dysrhythmia" [9], as suggested by both high-frequency [68] and low-frequency oscillations [69]. This proposed dysrhythmia may reduce thalamic control of the sensory cortices and render the pre-activation level low [33]. Thalamocortical dysrhythmia has been suggested in several diseases, e.g., tinnitus [70], neuropsychiatric disorders [71, 72] and chronic pain [73, 74]. However, in the present study, we found normal interictal LEP-habituation, although we observed deficient habituation and a tendency towards lower first-block amplitude of N1 during attacks, i.e., no tendency towards "normalization." Our present findings do not support the concept of a generally reduced interictal habituation in migraine.

On the other hand, the discrepancy between a preserved ictal N2P2-habituation, as opposed to a subtle deficient N1-habituation, suggests a centrally mediated ictal alteration [75]. The N1-component likely reflects the sensory-discriminative component of pain whereas the N2P2-component reflects the motivational and cognitive component of pain [59]. Thus, migraine pain seems to primarily affect sensory processes rather than cognitive, in contrast to the effects of sleep deprivation shown in one study [76].

In the present study, we could not reproduce altered N2P2-habituation or amplitude during attacks. This result contradicts the findings of other smaller studies. One study has shown reduced hand and face N2P2-habituation in interictal recordings (n = 14) compared to controls (n = 10) and a similar habituation deficit during attacks (n = 8) [21]. Two studies (n = 10 and 18) have demonstrated an increased N2P2-amplitude during compared to between attacks [23, 24]. Two of the studies included subjects with mean migraine frequency close to chronic migraine (we had none), and this could have contributed to the discrepancy between their and our results [21, 24].

The post hoc contrasts showing a lack of habituation of both N1 and N2P2-amplitudes in the postictal phase should be interpreted with caution as the number of postictal measurements were lower than for the other phases. Accordingly, this negative finding may be a result of rather low statistical power. Importantly, the main analyses showed no significant differences between habituation slopes, and Fig. 3 indicates that habituation is present in the postictal phase as well.

Pain scores increased linearly throughout the stimulation, in contrast to the decrease in N1 and N2P2-amplitudes. However, the negative correlations between

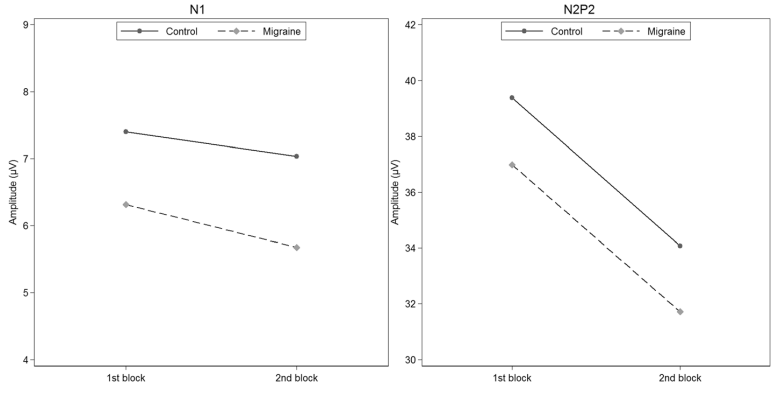

Fig. 4 Estimates of N1 (left) and N2P2-amplitude (right) habituation in interictal recordings and controls

Table 5 Estimated magnitudes and habituation in migraineurs in the interictal phase and controls

	N1 (μV)		N2P2 (μV)		Pain scores	
	Coef.	95% CI	Coef.	95% CI	Coef.	95% CI
Main effects						
Migraine	−1.014	[−3.767, 1.739]	−2.395	[−10.707, 5.917]	0.60	[−0.32, 1.52]
Habituation	−0.374	[−1.527, 0.778]	−5.307***	[−9.232, −1.383]	0.10	[−0.06, 0.26]
Interaction effect						
Migraine × Habituation	−0.268	[−1.875, 1.339]	−0.036	[−4.781, 4.854]	0.13	[−0.09, 0.35]
Constant	7.365	[5.070, 9.660]	39.382	[33.531, 45.234]	3.54	[3.01, 4.07]

The constant represents first-block amplitude or pain score responses in controls. The main effect of migraine represents the first-block amplitude or pain score difference between groups. The main effect of habituation represents the difference between first and second block amplitudes, or linear change in pain scores, in the control group. The interaction effect represents the habituation-difference between groups. Thus, the significant coefficient is interpreted as N2P2 habituation in the control group. The corresponding interaction effect is not significant, indicating no difference in habituation between controls and migraineurs in the interictal phase. Random effects estimates are shown in Supplementary Table 3. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

pain score and amplitudes were not significant. Mean pain scores and linear change of pain scores were not different between phases. Previous studies have shown reduced laser-pain thresholds during the attack [23, 24], and one study has shown increased pain scores during compared to between attacks by stimulation on both sides of the face but not the hands [21].

Habituation did not differ between migraineurs with and without aura. This finding cannot be compared to previous studies of LEP-habituation as they only included migraineurs without aura [17–22]. The positive correlation between pain scores and LEP amplitudes fits with earlier migraine studies [18–21, 24]. Interestingly, subjects with a bilateral headache had deficient postictal habituation compared to lateralized headache. We speculate if bilaterality represents excessive headache load, but a similar habituation deficiency was not observed for the load-parameter "years lived with migraine" (controlled for age, intensity, and frequency). However, *preictal* habituation was less pronounced in subjects with more migraine-years. Thus, there is some evidence of subtle changes of habituation by clinical features in proximity to attack, but the subgroups are small (e.g., only ten interictal and seven postictal sessions were associated with a bilateral headache), and the analyses many, hence it may be a random type 1 error.

Migraineurs in the interictal phase and controls showed no group differences. The amplitudes and pain scores were similar, and both groups had no significant N1-habituation but significant N2P2-habituation, and no linear change in pain scores. These findings are in contrast with some of the previously published results. The group differences in amplitude have varied considerably between studies. Group differences in N1 or N2P2-amplitudes after hand or face stimulation have only been reported in small studies (n = 9–14 in each group) [18–20]. There were no amplitude differences between groups in a larger study (n = 24 and 28) [17], including the present study (n = 29 and 30). In contrast to our results, one small study has reported habituation of N1-amplitudes in controls after hand stimulation compared to no habituation in migraineurs [20]. The same study showed no habituation in controls after face stimulation, but an extreme amplitude *potentiation* of more than 90% in migraineurs [20]. Valeriani et al. [17] showed N1-habituation after hand stimulation in controls (but not in migraineurs) and no habituation in either group after face stimulation. However, it is unclear if the migraine group had significantly reduced amplitude habituation compared to the control group because the authors did not compare the degree of habituation between groups statistically.

N2P2-habituation was reduced in migraineurs compared to controls after face [17–19, 21, 22] and hand [17, 18, 21, 22] stimulation in most previous studies, although one study showed no differences [20]. The reliability of significant effects in small studies is low even in the absence of other biases [77]. Independent replications are thus necessary to increase the reliability of the estimated effects. Based on the results of the present larger and blinded study, it seems reasonable to conclude that habituation and amplitudes after hand stimulation are not different in migraineurs compared to non-headache control subjects, or if they are different, the differences are small. It has been argued that deficient habituation is a neurophysiological hallmark of migraine [78, 79]. However, as for VEP [32], the contradictory findings of LEP studies do not support that hypothesis.

Pain score changes by stimulus repetitions did not differ between migraineurs and controls in the present study. Previous studies have demonstrated similar findings [18, 20], although one study found differences represented by pain score habituation in controls and potentiation in migraineurs [21]. Also, de Tommaso et al. [19] demonstrated pain score habituation in controls and only a habituation tendency in migraineurs, but they did compare the groups statistically.

Variation in applied methods may explain some of the discrepancy mentioned above (Table 6). For instance, three

Table 6 Studies on LEP-habituation in migraine

Author	Aim/ habituation measure		♀	♂	Age	Incl	Excl	Freq	Blinding	Laser	Intensity	Block	Stim	ISI	IBI	Comp	Sites	Main habituation results[a]
Valeriani 2003 (17)	Habituation/ % change	Mig:	14	10	33.7 ± 8.2	NR	NR	NR	NR/NR	CO_2	2.5 × STh	3	15–30[b]	8–12	5 m	N1 N2P2	Bilateral hands and face[c]	N1 hand: CO↘ Mig→
										10.6 μm								N1 face: CO → Mig→
		CO:	17	11	32.5 ± 6.4	NR	NR			2 mm								N2P2 hand: CO↘ > Mig↘
										10 ms								N2P2 face: CO↘ Mig→
de Tommaso 2005 (19)	Habituation/ ANOVA	Mig:	9	5	22–53	NR	NR	NR	NR/NR	CO_2	7.5 W	3	21	10	10s	N2P2	Rigth supraorbital	N2P2 face: CO↘ Mig→
										10.6 μm								
		CO:	7	3	21–50	NR	NR			2.5 mm								
										20 ms								
de Tommaso 2005 (21)	Interictal-ictal habituation/ ANOVA	Mig:	14	0	18–40	NR	NR	9.4 ± 4.6	NR/Yes	CO_2	7.5 W	3	20	10	10s	N2P2	Bilateral hands and supraorbital	N2P2 hand: CO↘ > Mig→
										10.6 μm								
		CO:	10	0	22–36	NR	NR			2.5 mm								N2P2 face: CO↘ > Mig→
										20 ms								
de Tommaso 2009 (18)	Pre-menstrual habituation/ Ratio	Mig:	9	0	26 ± 6.8	Outp	11	4 ± 2.94	NR/Yes	CO_2	7.5 W	3	20	10–15	5 m	N2P2	Right hand and supraorbital	N2P2 hand: CO > Mig
										10.6 μm								
		CO:	10	0	26.8 ± 5.3	Hosp staff	15			2 mm								N2P2 face: CO > Mig
										25 ms								
Di Clemente 2013 (20)	Intervention[d] / % change	Mig:	9	4	38.5 ± 12.0	NR	9	5.8 ± 2.2	NR/Yes	Nd:YAP	2.5 × STh	3	15	10	5 m	N1 N2P2	Right hand and supraorbital	N1 hand: CO > Mig
										NR								N1 face: CO > Mig
		CO:	10	5	30.9 ± 5.7	NR	0			NR								N2P2 hand: CO = Mig
										NR								N2P2 face: CO = Mig
Vecchio 2016 (22)	Intervention[e] / % change	Mig:	23	9	35.5 ± 10	NR	1	6 ± 2.5	NR/Yes	CO_2	PTh + 1 W	3	7	10	10s	N2P2	Right hand and supraorbital	N2P2 hand: CO > Mig
										10.6 μm								
		CO:	12	4	36.0 ± 11	NR	0			2 mm								N2P2 face: CO > Mig
										25 ms								
Uglem 2017	Habituation by phase/ Multilevel mixed mod.	Mig:	41	8	40 ± 10	Adv	Yes[f]	4[g]	Yes/Yes	Nd:YAP	3–5.5 J	2	21	6–10	10s	N1 N2P2	Right hand	N1 hand: CO → =Mig→
										1.34 μm								
		CO:	25	5	38 ± 11	Adv				8 mm								N2P2 hand: CO↘ = Mig↘
										6 ms								

♀: number of females. ♂: number of males. Age is presented in mean ± SD or range. Incl: inclution/method of recruitment. Excl: number of exclusions. Freq: migraine attack frequency in mean ± SD days/monts. Blinding: blinding of investigators during examination/blinding of investigators during LEP-analysis. Laser: Type of laser, wavelength, beam diameter and stimulus duration. Block: number of blocks. Stim: number of stimuli per block. ISI: interstimuli interval in seconds. IBI: interblock interval. Comp: analyzed LEP-components. Mig: migraine. CO: control. NR: not reported. Outp: outpatients. Hosp staff: hospital staff. Adv: recruited by advertisement. STh: sensory threshold. PTh: pain threshold. ↘: habituation present. →: no significant habituation. >: increased habituation in the control group compared to the migraineur group. =: no significant difference in habituation between the groups

[a]Only results regarding habituation of N1 and/or N2P2-amplitudes are shown. The studies differ in how they analyzed habituation as some reported habituation in each group separately (results indicated by ↘ or →), some reported comparisons between groups (results indicated by > or =), and some reported both. Valeriani 2003 (17) reported both results for N2P2 hand, but not for the other amplitudes.

[b]Three blocks with 15 stimuli at each side of the face and three blocks with 30 stimuli at each hand.

[c]Data were collected from three centers that stimulated two different face regions. The perioral region was stimulated in all migraineurs and 17 of the control subjects, while the supraorbital region was stimulated in 13 controls. The reported number of controls from each center does not add up to the reported total of 28 controls.

[d]The study examined the effect of topiramate on LEPs.

[e]The study investigated the effect of transcranial direct current stimulation of the left primary motor cortex and left dorsolateral prefrontal cortex on LEPs.

[f]Exclutions and dropouts are presented in Additional file 1: Table S1.

[g]Frequency was recorded in blocks. The three blocks 1–3, 4–7 and 8–14 days/month contained 14, 30 and 5 migraineurs, respectively.

of the previous studies recorded LEPs from three blocks with a five-minute break in between while we recorded two blocks without delay. Therefore, the less pronounced habituation shown by those studies may represent late effects only present after about ten minutes of stimulation. A similar late effect has been shown in radiculopathy patients where the habituation of N2P2 was normal in the first three blocks of 25 stimuli (inter-stimuli interval 8–12 s, no break between blocks), but deficient in the fourth [25].

Strengths and limitations

The major strengths of this study are its relatively large size with a longitudinal design and rigorous blinding both during data collection and analysis. The level of arousal, attention, and distraction may affect LEPs [60, 61]. Therefore, within-study consistency of the laser stimulation procedure is important. Especially when comparing groups, blinding of the investigators performing the stimulations becomes a necessity. Unfortunately, none of the previous migraine LEP-studies reported blinding of the investigators during stimulation, although the majority analyzed the LEPs blind to diagnosis (Table 6).

The solid-state laser used in this study differs from the CO_2-lasers employed in previous studies in that it produces a laser beam with shorter wavelength with deeper skin penetration that activates nociceptors more directly. This increases the amplitude of N1 and N2 and shortens the latency of N2P2, but the distribution of brain generators remains equal [80]. We do not believe that these differences, or the subtle differences in target-intensity, can explain the discrepancy between previous and present results.

The longitudinal design ensured a substantial number of interictal and preictal measurements and an acceptable number of ictal measurements. The postictal estimates are the least reliable due to the lowest number of measurements in that phase [77], although the number is comparable to previous migraine LEP-studies [21, 23, 24]. Only first-session responses were included in the migraineur versus control analyses because the investigator could not be blinded to diagnosis for the subsequent sessions, and to avoid possible long-term habituation/sensitization effects in later exams. Nevertheless, the number of interictal responses in this study was equal to [17, 22] or considerably larger than in the previous studies whose findings we attempted to reproduce [18–21].

We always stimulated the right hand regardless of the side the migraineur predominantly experienced headache. We found no habituation differences of LEPs obtained ipsilateral and contralateral to a migraine headache in accordance with previous findings [17]. Hence, it is seemingly not necessary to adjust the stimulated side according to headache laterality. We did not collect information on clinical allodynia, which could be of importance as an explanatory variable. We recruited both migraineurs and controls from the general population, and this design may enhance the generalizability of our results to the standard migraine population [81]. Having a first-degree relative that suffers from migraine may influence the habituation in controls [82]. However, we found habituation in both groups, not lack of habituation, which would be the expected finding if migraine-related genes biased our control group. Also, only four of the controls in our study had a positive family history of migraine, and excluding them from the analyses did not change the conclusions (results not reported).

The use of symptomatic treatment may have influenced the results as both triptans and non-steroidal anti-inflammatory drugs may reduce the amplitudes during the ictal phase [83]. It is unlikely that the medication influenced other phases than the ictal phase due to short half-life. Lack of facial stimulation can also be considered a limitation. However, hand and face LEP habituation seems to agree quite well in previous studies (Table 6), and this is to be expected as LEP reflects activation of a large part of the bilateral cortical pain network, and our aim was to study the generalized effects. Also, other modalities like pain thresholds have shown abnormalities in hands (and face) [84–86], suggesting an eventual thalamocortical dysfunction in migraine, in line with the development of cutaneous allodynia demonstrated by Burstein et al. [87]. Nevertheless, the present results are valid only for the more global pain function in migraine. It is necessary to do a similar study with face stimulation to conclude about lateralized second order trigeminal medullar afferent sensitization can be detected by LEP-abnormalities.

Previous studies have calculated habituation differently (Table 6). The method we chose included all available data and estimated the amplitude-change without prior calculation/manipulation of the dependent variable. Also, the approach did not use listwise deletion of cases with missing values, as would be the case with ANOVA. We were thus able to compare all four phases in one model. We also included the N1-responses where the signal to noise ratio was too low, as interval censored variables instead of discarding them, to avoid exclusion bias [65].

Conclusion

Both imaging and neurophysiological studies have shown phasic alterations in migraineurs. However, we only found evidence of a subtle alteration of habituation of cerebral responses to painful laser stimulation in the ictal phase. We found comparable LEP-amplitudes and habituation to dorsal hand stimulation in migraineurs in the interictal phase and headache-free controls. Thus, in contrast to some previous studies, we conclude that cerebral responses to painful laser stimulation are normal interictally in migraineurs. LEPs seem to be stable throughout the migraine cycle, but we could not exclude small changes and recommend further studies on phase-related changes in pain-physiology.

Additional file

Additional file 1: Table S1. Number of migraineurs and controls at each recruitment stage. **Table S2.** Estimated magnitudes and habituation of N1, N2P2 and pain scores by phase. **Table S3.** Estimated magnitudes and habituation in migraineurs in the interictal phase and controls. **Table S4.** Estimated amplitudes and habituation of N1 and N2P2 by phase and the effect of aura. **Table S5.** Estimated amplitudes and habituation of N1 and N2P2 by phase and the effect of headache laterality. **Table S6.** Estimated amplitudes and habituation of N1 and N2P2 by phase and the effect of years lived with migraine (YwM). **Table S7.** Estimated amplitudes and habituation of N1 and N2P2 by phase and the effect of pain scores. (DOCX 39 kb)

Abbreviations
CI: Confidence interval; LEP: Laser-evoked potentials; NRS: Numerical rating scale; VEP: Visual evoked potentials

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Availability of data and materials
Further data from the underlying research material can be obtained upon request to the corresponding author.

Authors' contributions
MU wrote the first draft. PMO, TS, and MU did major revisions. All authors contributed to the final revision. All authors contributed to the planning of the study, supervised by TS. MS and GBG were responsible for inclusion, coordination, and follow-up of participants. Examinations, data processing, and statistics were done by MU, supervised by PMO and TS. All authors read and approved the final manuscript.

Ethics approval and consent to participate
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.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

Author details
[1]Department of Neuromedicine and Movement Science, NTNU, Norwegian University of Science and Technology, Trondheim, Norway. [2]Department of Neurology and Clinical Neurophysiology, St. Olavs Hospital, Trondheim, Norway. [3]Norwegian Advisory Unit on Headaches, St. Olavs Hospital, Trondheim, Norway. [4]NTNU, Faculty of Medicine and Health Sciences, P.B. 8905, N-7491 Trondheim, Norway.

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Additional file 1

Table S1. Number of migraineurs and controls at each recruitment stage.

	Migraine			Controls		
	N	**Excl.**	**Dropout**	***N***	**Excl.**	**Dropout**
Telephone screening	74	6	13	40	2	7
Inclusion by neurologist	55	2	3			
1st exam	50	1	1	31	1	
2nd exam	48		1			
3rd exam	47		1			
4th exam	46					

One migraineur withdrew consent after the first exam and was excluded. One control was excluded because we were unable to obtain reliable LEPs as most trials were rejected. Drop outs account for six missing tests in migraineurs. *N:* number of subjects. Excl.: number of excluded subjects. Dropout: number of subjects who dropped out due to personal reasons.

Statistical details

Migraine phase and block number were dummy-coded with the interictal phase and first block as a baseline when analyzing N1 and N2P2. Phase, block, and their interactions were included as fixed factors. Thus, the constant represented the mean interictal first-block amplitudes, and the main effects represented the difference in first-block amplitudes from the interictal to the preictal, ictal, and postictal phases, and the interictal amplitude change from the first to the second block. The interaction effects represented differences in change from the first to the second block from the interictal to the preictal, ictal and postictal phases.

Dependencies in the data were accounted for by including subject and session (1,2,3 or 4) as random factors in the first set and subject as a random factor in the second set of the multilevel models. Thus, the first set was modeled as a three-level model with responses nested in sessions nested in subjects, and the second set as a two-level model with responses nested in subjects. The random slope of time was included in the analysis of pain scores. Normality of residuals and random factors were controlled with histograms. N1 and N2P2 in both sets were square rooted to improve normality. A robust variance covariance estimator was applied.

The extended models were extensions of the original models with three-way interactions between the included variables and all two-way interactions.

Table S2. Estimated magnitudes and habituation of N1, N2P2 and pain scores by phase.

	N1 ($\mu V^{0.5}$)		N2P2 ($\mu V^{0.5}$)		Pain scores	
	Coef.	**95% CI**	**Coef.**	**95% CI**	**Coef.**	**95% CI**
Main effects						
Preictal	0.171	[-0.083, 0.425]	0.110	[-0.162, 0.381]	-0.01	[-0.36, 0.34]
Ictal	-0.123	[-0.339, 0.092]	0.089	[-0.233, 0.411]	0.23	[-0.28, 0.74]
Postictal	0.153	[-0.085, 0.391]	0.197	[-0.192, 0.585]	0.37	[-0.35, 1.10]
Habituation	-0.126*	[-0.251, -0.001]	-0.306***	[-0.434, -0.178]	0.21***	[0.09, 0.33]
Interaction effects						
Preictal × Habituation	-0.157	[-0.416, 0.102]	-0.037	[-0.283, 0.209]	0.07	[-0.10, 0.24]
Ictal × Habituation	0.054	[-0.149, 0.256]	-0.034	[-0.338, 0.269]	0.06	[-0.18, 0.31]
Postictal × Habituation	-0.199	[-0.547, 0.148]	0.071	[-0.288, 0.429]	0.12	[-0.17, 0.41]
Constant	2.662	[2.493, 2.832]	6.065	[5.753, 6.377]	4.11	[3.56, 4.67]
Random effects						
Level 3: subject (intercept)		0.178		1.014		3.31
Level 2: session (intercept)		0.045		0.170		0.86
Level 2: time (slope)						0.11
Level 1: residuals		0.156		0.207		1.90

N1 and N2P2-amplitudes were square root transformed to improve normality of residuals and coefficient magnitudes should be interpreted accordingly. Results in the paper are presented in the original scale. The constant represents interictal first-block or mean pain score responses, the first three main effects are first-block amplitude or pain score differences from the interictal phase and the fourth "Habituation" main effect is the difference between first and second block, or the linear change of pain scores, in the interictal phase. The interaction effects represent habituation differences between the interictal phase and the preictal, ictal and postictal phases, respectively. Thus, the significant coefficients are interpreted as decreased second-block N1 and N2P2-amplitudes, and linear increase in pain scores, in the interictal phase, i.e. interictal N1 and N2P2 habituation and subjective pain sensitization. Lack of significant interaction effects are interpreted as no habituation differences between the interictal phase and the other phases. Random effect estimates are displayed as variances. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table S3. Estimated magnitudes and habituation in migraineurs in the interictal phase and controls.

	N1 ($\mu V^{0.5}$)		N2P2 ($\mu V^{0.5}$)		Pain scores	
	Coef.	**95% CI**	**Coef.**	**95% CI**	**Coef.**	**95% CI**
Main effects						
Migraine	-0.181	[-0.667, 0.305]	-0.194	[-0.867, 0.479]	0.60	[-0.32, 1.52]
Habituation	-0.065	[-0.264, 0.134]	-0.438***	[-0.760, -0.117]	0.10	[-0.06, 0.26]
Interaction effect						
Migraine × Habituation	-0.056	[-0.343, 0.232]	-0.012	[-0.408, 0.384]	0.13	[-0.09, 0.35]
Constant	2.892	[2.496, 3.289]	6.276	[5.809, 6.742]	3.54	[3.01, 4.07]
Random effects						
Level 2: subject (intercept)		0.214		1.339		3.06
Level 2: time (slope)						0.07
Level 1: residuals		0.111		0.283		2.22

N1 and N2P2-amplitudes were square root transformed to improve normality of residuals and coefficient magnitudes should be interpreted accordingly. The constant represents first-block amplitude or pain score responses in controls. The main effect of migraine represents the first-block amplitude or pain score difference between groups. The main effect of habituation represents the difference between first and second block amplitudes, or linear change in pain scores, in the control group. The interaction effect represents the habituation-difference between groups. Thus, the significant coefficient is interpreted as N2P2 habituation in the control group. The corresponding interaction effect is not significant, indicating no difference in habituation between controls and interictal migraineurs. Random effect estimates are displayed as variances. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table S4. Estimated amplitudes and habituation of N1 and N2P2 by phase and the effect of aura.

	N1 ($\mu V^{0.5}$)		N2P2 ($\mu V^{0.5}$)	
	Coef.	**95% CI**	**Coef.**	**95% CI**
Main effects				
Preictal	0.188	[-0.142, 0.518]	0.245	[-0.116, 0.605]
Ictal	-0.193	[-0.488, 0.102]	0.074	[-0.348, 0.497]
Postictal	0.031	[-0.228, 0.291]	0.145	[-0.336, 0.627]
Habituation	-0.065	[-0.197, 0.067]	-0.286***	[-0.445, -0.128]
Aura	0.199	[-0.131, 0.530]	0.218	[-0.439, 0.876]
Two-way interaction effects				
Preictal × Habituation	-0.201	[-0.555, 0.152]	0.038	[-0.287, 0.364]
Ictal × Habituation	0.102	[-0.178, 0.382]	0.000	[-0.400, 0.400]
Postictal × Habituation	-0.124	[-0.439, 0.192]	0.046	[-0.402, 0.494]
Preictal × Aura	-0.063	[-0.578, 0.451]	-0.324	[-0.875, 0.228]
Ictal × Aura	0.134	[-0.263, 0.531]	-0.021	[-0.677, 0.636]
Postictal × Aura	0.273	[-0.180, 0.726]	0.177	[-0.639, 0.993]
Habituation × Aura	-0.174	[-0.448, 0.099]	-0.060	[-0.333, 0.212]
Three-way interaction effects				
Preictal × Habituation × Aura	0.137	[-0.391, 0.665]	-0.153	[-0.660, 0.353]
Ictal × Habituation × Aura	-0.077	[-0.444, 0.291]	-0.067	[-0.691, 0.557]
Postictal × Habituation × Aura	-0.161	[-0.928, 0.607]	0.061	[-0.696, 0.819]
Constant	2.592	[2.370, 2.814]	5.989	[5.595, 6.382]
	Estimate		**Estimate**	
Random effects				
Level 3: subject	0.173		1.042	
Level 2: session	0.046		0.166	
Level 1: residuals	0.152		0.211	

N1 and N2P2-amplitudes were square root transformed to improve normality of residuals and coefficient magnitudes should be interpreted accordingly. The constant represents interictal first-block responses in subjects with migraine without aura. None of the three-way interactions were significant, that is, habituation-differences between phases were not different between migraineurs with and without aura. Interictal first-block amplitudes and habituation were not different between migraineur with and without aura. Random effect estimates are displayed as variances. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table S5. Estimated amplitudes and habituation of N1 and N2P2 by phase and the effect of headache laterality.

	N1 ($\mu V^{0.5}$)		N2P2 ($\mu V^{0.5}$)	
	Coef.	**95% CI**	**Coef.**	**95% CI**
Main effects				
Preictal	0.043	[-0.419, 0.505]	0.119	[-0.425, 0.662]
Ictal	-0.085	[-0.473, 0.303]	0.355	[-0.280, 0.990]
Postictal	-0.063	[-0.692, 0.567]	0.562	[-0.384, 1.508]
Habituation	-0.028	[-0.221, 0.165]	-0.336**	[-0.551, -0.120]
Left side	0.121	[-0.222, 0.464]	-0.001	[-0.538, 0.536]
Bilateral	-0.152	[-0.698, 0.393]	0.161	[-0.583, 0.905]
Two-way interaction effects				
Preictal × Habituation	-0.150	[-0.612, 0.313]	0.077	[-0.355, 0.509]
Ictal × Habituation	-0.195	[-0.459, 0.069]	-0.164	[-0.671, 0.343]
Postictal × Habituation	-0.231	[-0.998, 0.537]	-0.292	[-1.072, 0.487]
Preictal × Left side	0.162	[-0.549, 0.873]	-0.224	[-1.185, 0.737]
Preictal × Bilateral	0.342	[-0.502, 1.187]	0.084	[-0.784, 0.952]
Ictal × Left side	-0.031	[-0.617, 0.555]	-0.029	[-1.132, 1.073]
Ictal × Bilateral	-0.051	[-0.684, 0.581]	-0.886	[-1.939, 0.167]
Postictal × Left side	0.245	[-0.407, 0.898]	-0.196	[-1.346, 0.955]
Postictal × Bilateral	0.427	[-0.445, 1.298]	-0.764	[-2.052, 0.523]
Habituation × Left side	-0.170	[-0.429, 0.089]	0.113	[-0.193, 0.418]
Habituation × Bilateral	0.168	[-0.300, 0.636]	-0.356	[-0.835, 0.123]
Three-way interaction effects				
Preictal × Habituation × Left side	-0.012	[-0.516, 0.491]	-0.087	[-0.682, 0.508]
Preictal × Habituation × Bilateral	-0.283	[-1.209, 0.644]	0.016	[-0.718, 0.749]
Ictal × Habituation × Left side	0.344	[-0.170, 0.857]	-0.098	[-0.862, 0.667]
Ictal × Habituation × Bilateral	0.143	[-0.440, 0.727]	0.822	[-0.003, 1.647]
Postictal × Habituation × Left side	-0.074	[-0.966, 0.819]	0.121	[-0.909, 1.150]
Postictal × Habituation × Bilateral	-0.174	[-1.288, 0.940]	1.029*	[0.014, 2.045]
Constant	2.644	[2.367, 2.921]	-0.814	[-1.046, -0.582]
	Estimate		**Estimate**	
Random effects				
Level 3: subject	0.198		0.998	
Level 2: session	0.044		0.196	
Level 1: residuals	0.144		0.219	

N1 and N2P2-amplitudes were square root transformed to improve normality of residuals and coefficient magnitudes should be interpreted accordingly. Headache laterality was classified by the related attack if the phase was preictal, ictal or postictal. Interictal recordings were classified by the side the subject most commonly experienced headache, either left, right or bilateral. Sixteen interictal recordings had an equal amount of left and right-sided unilateral migraine and were not included in this analysis. The constant represents interictal first-block responses in subjects with right-sided migraine, the same side as the laser-stimuli were applied. Subjects with bilateral migraine had reduced N2P2-habituation (more positive slope) in the postictal phase compared to the interictal phase, and the same tendency was present in the ictal phase. Habituation was not different between left and right-sided migraine. Headache laterality did not significantly affect interictal estimates of first-block amplitude and habituation. Random effect estimates are displayed as variances. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table S6. Estimated amplitudes and habituation of N1 and N2P2 by phase and the effect of years lived with migraine (YwM).

	N1 ($\mu V^{0.5}$)		N2P2 ($\mu V^{0.5}$)	
	Coef.	**95% CI**	**Coef.**	**95% CI**
Main effects				
Preictal	0.136	[-0.092, 0.365]	0.082	[-0.197, 0.362]
Ictal	-0.124	[-0.341, 0.092]	0.098	[-0.223, 0.419]
Postictal	0.082	[-0.157, 0.320]	0.141	[-0.252, 0.533]
Habituation	-0.124*	[-0.246, -0.002]	-0.307***	[-0.433, -0.180]
YwM	-0.016	[-0.034, 0.003]	-0.002	[-0.039, 0.036]
Two-way interaction effects				
Preictal × Habituation	-0.145	[-0.394, 0.104]	-0.013	[-0.257, 0.231]
Ictal × Habituation	0.053	[-0.149, 0.255]	-0.042	[-0.343, 0.259]
Postictal × Habituation	-0.120	[-0.407, 0.167]	0.103	[-0.259, 0.464]
Preictal × YwM	-0.012	[-0.046, 0.022]	-0.020	[-0.056, 0.016]
Ictal × YwM	0.007	[-0.011, 0.025]	0.025	[-0.009, 0.058]
Postictal × YwM	-0.012	[-0.052, 0.028]	-0.023	[-0.068, 0.022]
Habituation × YwM	0.005	[-0.010, 0.020]	-0.005	[-0.018, 0.008]
Three-way interaction effects				
Preictal × Habituation × YwM	0.009	[-0.018, 0.036]	0.037*	[0.009, 0.066]
Ictal × Habituation × YwM	-0.011	[-0.028, 0.006]	0.014	[-0.017, 0.046]
Postictal × Habituation × YwM	0.029	[-0.027, 0.085]	0.024	[-0.019, 0.066]
Control variables				
Age	0.000	[-0.014, 0.014]	-0.038*	[-0.070, -0.005]
Migraine intensity 1	-0.525	[-1.082, 0.032]	-0.539	[-2.093, 1.015]
Migraine intensity 3	-0.186	[-0.413, 0.042]	-0.336	[-0.942, 0.269]
Migraine frequency 1	0.130	[-0.152, 0.413]	0.130	[-0.529, 0.789]
Migraine frequency 3	0.398*	[0.049, 0.747]	0.980	[-0.025, 1.986]
Constant	2.715	[2.481, 2.949]	6.142	[5.662, 6.621]
	Estimate		**Estimate**	
Random effects				
Level 3: subject	0.139		0.898	
Level 2: session	0.046		0.167	
Level 1: residuals	0.152		0.203	

N1 and N2P2-amplitudes were square root transformed to improve normality of residuals and coefficient magnitudes should be interpreted accordingly. Years with migraine (YwM) and age were centered at their means. Migraine intensity and frequency were dummy-coded with category 2 as base in both, i.e. moderate intensity and 4-7 days with migraine/month (intensity: 1: mild, 2: moderate, 3: severe, and frequency: 1: 1-3 days/month, 2: 4-7 days/month, 3: 8-14 days/month). The constant represents interictal first-block responses at mean YwM, mean age, migraine intensity = 2, and migraine frequency = 2. Subjects who have lived more years with migraine have decreased N2P2-habituation in the preictal phase compared to the interictal phase. First-block amplitude and habituation in the interictal phase did not differ with YwM. Random effect estimates are displayed as variances. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table S7. Estimated amplitudes and habituation of N1 and N2P2 by phase and the effect of pain scores.

	N1 ($\mu V^{0.5}$)		N2P2 ($\mu V^{0.5}$)	
	Coef.	**95% CI**	**Coef.**	**95% CI**
Main effects				
Preictal	0.195	[-0.045, 0.435]	0.134	[-0.116, 0.384]
Ictal	-0.138	[-0.338, 0.063]	0.091	[-0.206, 0.388]
Postictal	0.157	[-0.035, 0.348]	0.121	[-0.270, 0.512]
Habituation	-0.129*	[-0.252, -0.006]	-0.312***	[-0.435, -0.188]
Pain scores	0.092*	[0.014, 0.170]	0.219***	[0.142, 0.295]
Two-way interaction effects				
Preictal × Habituation	-0.170	[-0.417, 0.077]	-0.084	[-0.324, 0.156]
Ictal × Habituation	0.068	[-0.142, 0.277]	-0.051	[-0.350, 0.247]
Postictal × Habituation	-0.210	[-0.506, 0.087]	-0.117	[-0.512, 0.278]
Preictal × Pain scores	-0.024	[-0.131, 0.083]	-0.031	[-0.159, 0.097]
Ictal × Pain scores	0.014	[-0.095, 0.122]	0.010	[-0.149, 0.169]
Postictal × Pain scores	-0.020	[-0.120, 0.081]	0.003	[-0.141, 0.146]
Habituation × Pain scores	-0.005	[-0.065, 0.056]	-0.028	[-0.088, 0.032]
Three-way interaction effects				
Preictal × Habituation × Pain scores	-0.018	[-0.152, 0.116]	0.009	[-0.119, 0.137]
Ictal × Habituation × Pain scores	-0.063	[-0.172, 0.046]	-0.073	[-0.225, 0.079]
Postictal × Habituation × Pain scores	-0.008	[-0.165, 0.150]	0.089	[-0.052, 0.229]
Constant	2.652	[2.486, 2.817]	6.039	[5.719, 6.359]
	Estimate		**Estimate**	
Random effects				
Level 3: subject	0.173		1.118	
Level 2: session	0.037		0.119	
Level 1: residuals	0.151		0.193	

N1 and N2P2-amplitudes were square root transformed to improve normality of residuals and coefficient magnitudes should be interpreted accordingly. Pain scores were measured by a numerical rating scale (NRS) ranging from 0 = no pain to 10 = unbearable pain. Pain scores were centered at NRS = 4 before analysis. Thus, the constant represents interictal first-block responses at NRS = 4. There were no phase-differences in habituation by pain scores (none of the three-way interactions were significant). Pain scores did not affect habituation in the interictal phase, as shown by the "Habituation × Pain scores" interaction. Interictal N1 and N2P2 first-block amplitudes increased with increasing pain scores, as shown by the main effects of pain scores. Random effect estimates are displayed as variances. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.