Non-invasive cortical modulation of experimental pain in migraine

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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 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 et al. , 2012, Noseda et al. , 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 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 et al. , 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).

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.

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. , 2016).

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 (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 et al. , 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 minutes 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 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 x 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 seconds between-trains intervals. An rTMS-session lasted for 4 minutes and 20 seconds. 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). 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 x 50 mm Peltier element thermode (Somedic Sales AB, Stockholm). 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 seconds 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 seconds duration. Degree of pain was reported continuously. The pain at 30 seconds 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) was used to run separate multilevel linear mixed-effects models (Rabe-Hesketh et al. , 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 (Figure 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 ($p < 0.005$, Figure 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.

3.3 Suprathreshold heat pain ratings

Pain ratings from both sites decreased in both groups after rTMS compared to baseline ($p < 0.005$, Figure 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. , 2016) 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. , 2016). 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. , 2016).

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, Brighina et al. , 2010, Brighina et al. , 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 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 seconds 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 seconds may predominantly represent differences in C fiber activity. Furthermore, rTMS did not alter maximal pain ratings (data not reported), generally occurring earlier than 30 seconds 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. (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. (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 et al. (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. , 2016).

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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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.

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)

Figure legends

Figure 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.

Figure 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.

Figure 3. Adjusted predictions of pain ratings after 30 seconds 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.
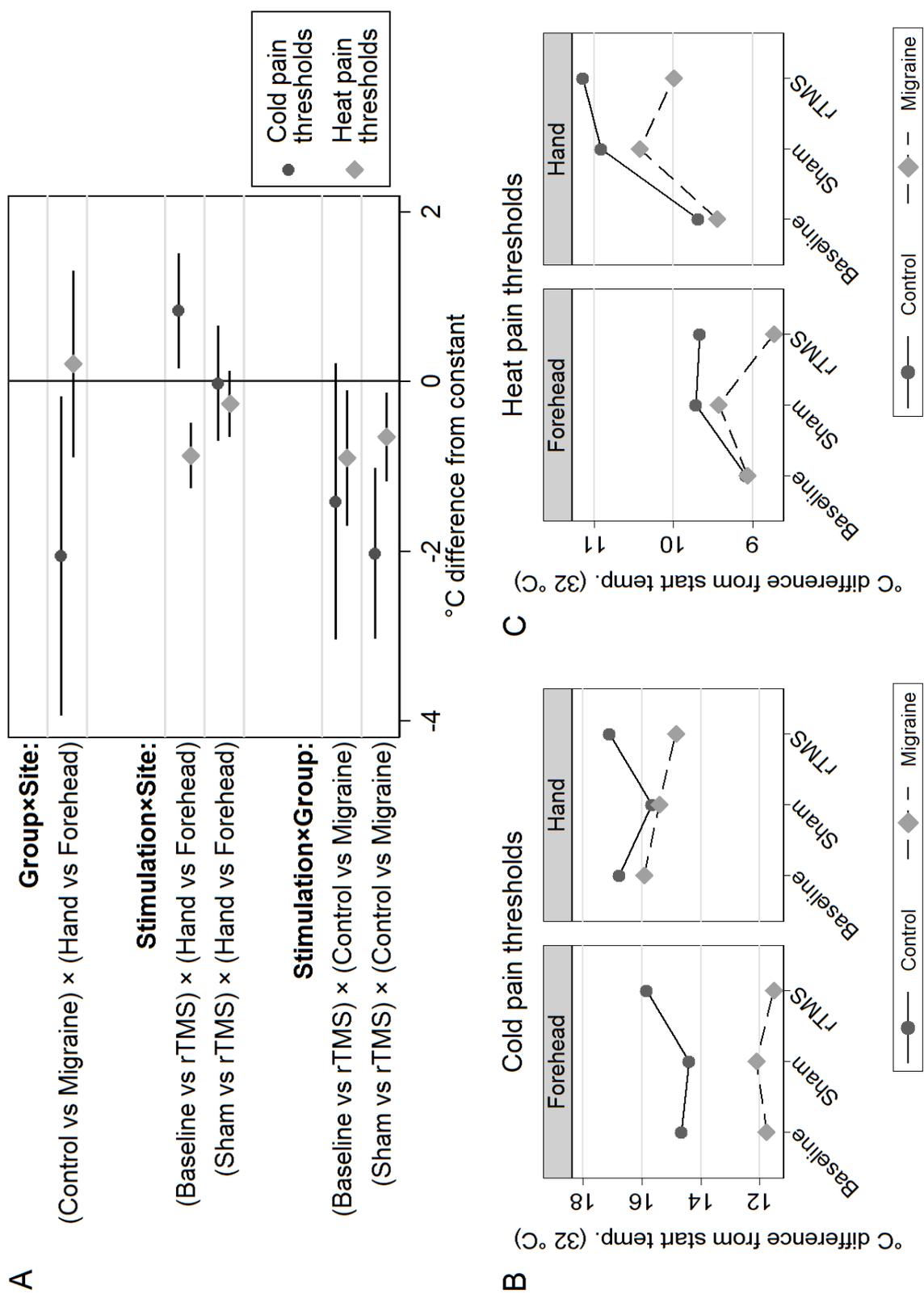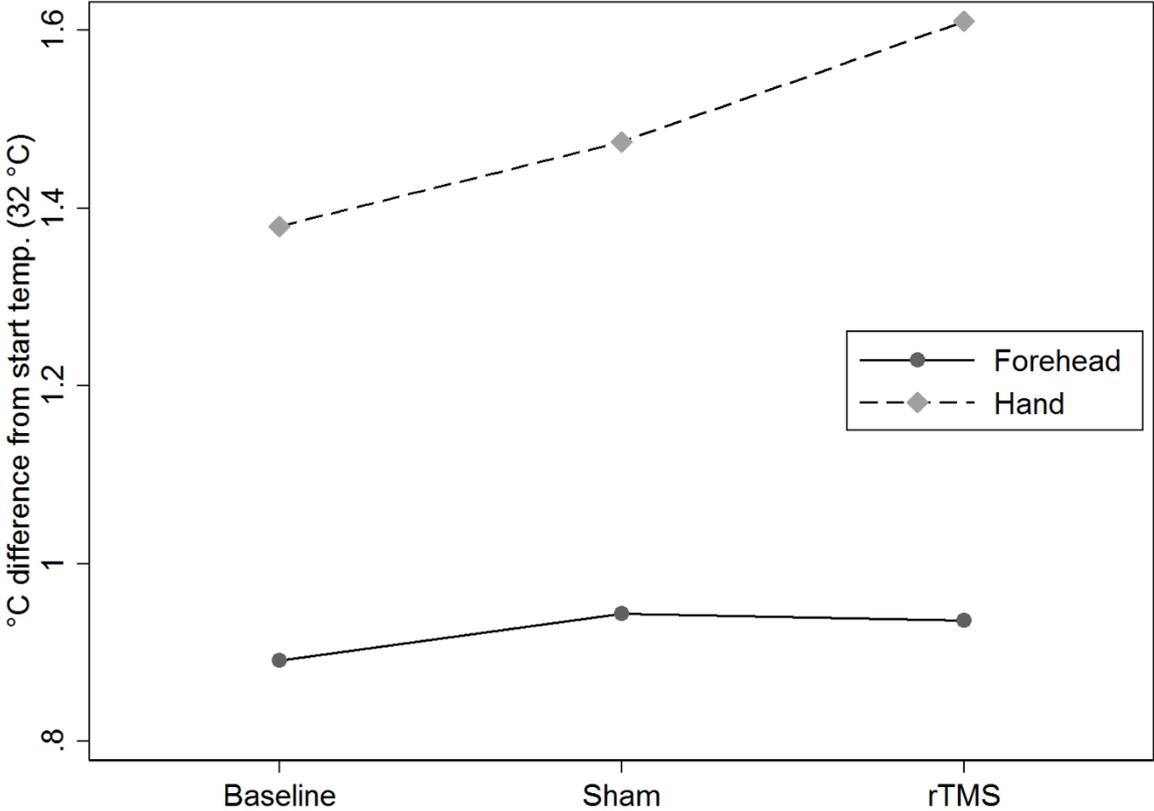
Figure 1

Figure 2

Figure 3