Clinical Neurophysiology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

Modulation of visual evoked potentials by high-frequency repetitive transcranial magnetic stimulation in migraineurs

Petter M. Omland [a,*], Martin Uglem [a], Morten Engstrøm [a,b], Mattias Linde [a,b], Knut Hagen [a,b], Trond 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

ARTICLE INFO

Article history:
Accepted 29 January 2014
Available online xxxx

Keywords:
Visual evoked potentials
Habituation
Migraine
Repetitive transcranial magnetic stimulation
Phosphene thresholds

HIGHLIGHTS

- High-frequency repetitive transcranial magnetic stimulation (rTMS) reduced visual evoked potential (VEP) habituation in migraineurs, indicating an increased cortical responsiveness to rTMS.
- The increased responsiveness to rTMS in migraineurs may be caused by a cortical dysfunction that changes in the period before a migraine attack.
- High-frequency rTMS stimulation did not restore VEPs to normal in migraineurs.

ABSTRACT

Objective: High-frequency repetitive transcranial magnetic stimulation (rTMS) modulates cortical excitability. We investigated its effect on visual evoked potentials (VEPs) in migraine.
Methods: Thirty-two headache-free controls (CO), 25 interictal (MINT) and 7 preictal migraineurs (MPRE) remained after exclusions. VEPs to 8′ and 65′ checks were averaged in six blocks of 100 single responses. VEPs were recorded before, directly after and 25 min after 10 Hz rTMS. The study was blinded for diagnosis during recording and for diagnosis and block number during analysis. First block amplitudes and habituation (linear amplitude change over blocks) were analysed with repeated measures ANOVA.
Results: With 65′ checks, N70-P100 habituation was reduced in MINT compared to CO after rTMS ($p = 0.013$). With 8′ checks, habituation was reduced in MPRE compared to MINT and CO after rTMS ($p < 0.016$). No effects of rTMS on first block amplitudes were found.
Conclusion: RTMS reduced habituation only in migraineurs, indicating increased responsivity to rTMS. The magnocellular visual subsystem may be affected interictally, while the parvocellular system may only be affected preictally.
Significance: Migraineurs may have increased responsiveness to rTMS because of a cortical dysfunction that changes before a migraine attack.

1. Introduction

Functional alterations in the migraine cortex may contribute to the development of migraine attacks (Vecchia and Pietrobon, 2012). It has been suggested that the visual cortex in migraineurs is either hyper- or hypoexcitable (Aurora and Wilkinson, 2007; Coppola et al., 2007b), and that the regulation of cortical excitability is altered in migraineurs (Antal et al., 2008).

Visual evoked potential (VEP) amplitude and VEP habituation depend on cortical excitability (Coppola et al., 2007b), but the mechanisms are complex and not fully understood (Coppola et al., 2007b; Rankin et al., 2009). Interictal migraineurs (MINT) may lack habituation. VEP amplitudes decrease during continuous stimulation in CO, but increase or remain stable in MINT (Coppola et al., 2009). However, this finding is controversial because it has not been reproduced in many studies (Afra et al., 2000a; Hansen et al., 2011; Khalil et al., 2000; Oelkers-Ax et al., 2005; Oelkers et al., 1999; Omland et al., 2013; Sand et al., 2008).

* Corresponding author. Address: Department of Neuroscience, Medisinsk teknisk forskningssenter, N-7491 Trondheim, Norway. Tel.: +47 93 24 43 52; fax: +47 73 59 87 95.
E-mail address: pmomland@gmail.com (P.M. Omland).

http://dx.doi.org/10.1016/j.clinph.2014.01.028
1388-2457/

The visual system can roughly be divided into the magno- and parvocellular subsystems. The magnocellular system mainly continues in the dorsal pathway of the parietal cortex, which is responsible for the visual guidance of movement (Kandel et al., 2013). The magnocellular subsystem is more sensitive to lower spatial frequency (i.e. VEPs with larger checks) than the parvocellular subsystem (Bassi and Lehmkuhle, 1990; Livingstone and Hubel, 1988). Studies applying both small and large check sizes only found differences in visual responses between migraineurs and CO with the larger check size (Chen et al., 2009; Sand et al., 2009). In addition, deficits of visual motion processing have been reported in migraineurs (McKendrick et al., 2001). The alterations in excitability of the visual cortex may therefore primarily involve the magnocellular subsystem (Chen et al., 2009).

Repetitive transcranial magnetic stimulation (rTMS) can modulate cortical excitability (Pascual-Leone et al., 1994). In one study, high-frequency rTMS restored normal VEP habituation in MINT, but had little effect in CO (Bohotin et al., 2002). However, this result has as far as we know not been reproduced with a blinded study design or a control group matched for age. To our knowledge, the effect of high-frequency rTMS on VEPs in MINT has only been investigated with small 8′ check stimuli (Bohotin et al., 2002) and not with larger (e.g. 65′) checks.

Migraineurs often experience symptoms in the preictal period, including increased sensitivity to light (Giffin et al., 2003). These prodromal symptoms may be related to altered cortical activity (Noseda and Burstein, 2013). Longitudinal studies have reported preictal changes in VEPs (Sand et al., 2008) and in cortical activity measured by quantitative electroencephalography (QEEG) (Bjork et al., 2011). The effect of high-frequency rTMS on VEPs has not been studied previously in preictal migraineurs (MPRE).

The aim of the present study was to compare the effect of high-frequency rTMS on VEPs in MINT, MPRE and CO with a blinded procedure. We hypothesized that the rTMS-induced changes in interictal migraineurs would be more pronounced with large 65′ than small 8′ checks, and that the changes would increase in the preictal period. For small checks, we wanted to confirm that the possibly abnormal VEPs in MINT could be changed and restored to normal by high-frequency rTMS.

2. Methods

2.1. Subjects

Subjects were recruited by intranet advertisement within our university. Subjects were both students and university employees (professors, nurses, technical staff, kindergarten staff, secretaries etc.). Forty-three migraineurs were included by neurologists according to the ICHD-II criteria (Headache Classification Committee of the International Headache Society, 2004). Thirty-four headache-free controls (CO) matched for sex and age were also recruited. Migraineurs with 2–6 migraine attacks and at most 10 migraine days per month were included. They kept a headache diary 4 weeks before and after the examination and completed a headache questionnaire. Migraineurs were classified according to migraine subtype and period: Interictal migraineurs without aura (MwoA, $n = 14$ after exclusions, see below), interictal migraineurs with aura (aura in at least 50% of attacks, MA, $n = 11$ after exclusions), migraineurs in the interictal period (MINT, $n = 25$, merging MwoA and MA) and migraineurs in the preictal period (<48 h before attack, MPRE, $n = 7$ after exclusions). There were too few patients in the headache phase ($n = 4$) and postictal period ($n = 1$, <48 h after attack), and these groups were therefore not included in further analysis. RTMS was not performed on the first migraineur (MA) and two first CO because of technical difficulties. Three MwoA were excluded prior to analysis, one because of a temporary pain condition during the examination and two because of drowsiness. Two MPRE were excluded because of drowsiness. Exclusions were made without knowledge of subjects' diagnosis. Demographical data of the participants are reported in Table 1.

Exclusion criteria were coexisting frequent episodic ($\geqslant$1–14 days/month for CO and $\geqslant$7–14 for migraineurs) or chronic ($\geqslant$15 days/month) tension-type headache, neurological or psychiatric diseases, first-degree relatives with epilepsy, 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, neuroleptic drugs, anti-depressive drugs, anti-epileptic drugs, prophylactic allergy treatment or other drugs which may influence neuronal, vascular or muscular function, alcohol or drug abuse, or ferromagnetic implants. Symptomatic medication during migraine attacks was allowed.

Investigators were blinded to diagnosis and had not met the subjects before the examination. All participants signed an informed consent form. The study was approved by the Regional Committee for Medical Research Ethics.

2.2. Procedure

Examinations were conducted at the same time of day in all subjects.

2.2.1. Visual evoked potentials

Visual acuity was measured on Snellens' chart. The VEP examination was performed in a quiet room with dimmed light (5 lux). Subjects were sitting in a relaxed position with eyes 100 cm from the screen. All subjects confirmed that they could see both the small and the large VEP checks clearly at this distance. The same instructions were given to all subjects before each VEP recording. They were instructed to relax and to focus on the fixation point in the middle of the checkerboard pattern. Only the right eye was tested. The left eye was covered with an eye-patch. A Viking Select® system (Nicolet Biomedical Inc., Madison, WI USA) was used for VEP recording. Checkerboard patterns were presented on a NIC-2015® visual stimulator (Nicolet Biomedical Inc., Madison, WI, USA) with $17 \times 13^{\circ}$ visual field and 93% contrast. Responses were recorded from the mid-occipital (MO, 5 cm above

Table 1
Demographic and clinical data for healthy controls and migraine patients after exclusions (Mean ± SD or no).

	CO	MINT	MPRE
No	32	25	7
Age	30.2 (10.4)	26.8 (8.2)	27.3 (9.3)
Women/Men	28/4	22/3	7/0
Visual acuity	1.0 (0.2)	1.1 (0.2)	1.1 (0.1)
MwoA/MA	NA	14/11	3/4
Headache-history (years)[1]	NA	12.8 (7.6)	10.6 (5.5)
Headache-frequency (1–4)[2]	NA	1.5 (0.6)	1.6 (0.5)
Headache-intensity (1–4)[3]	NA	2.6 (0.6)	2.7 (0.5)
Headache-days/month last 3 months	NA	4.3 (2.2)	4.6 (2.8)
Usual headache-attack duration (hours)	NA	11.5 (14.6)	13.0 (8.4)

NA: Not applicable. CO: Healthy controls. MINT: Interictal migraine. MPRE: Preictal migraine.
[1] Headache-history: Years since first appearance of headache.
[2] Headache-frequency: Number of headache days/month, 0: <1. 1: 1–3/month. 2: 4–7/month. 3: 8–14/month. 4: >14/month.
[3] Headache-intensity: 1: Mild. 2: Moderate. 3: Severe. 4: Extreme.

Please cite this article in press as: Omland PM et al. Modulation of visual evoked potentials by high-frequency repetitive transcranial magnetic stimulation in migraineurs. Clin Neurophysiol (2014), http://dx.doi.org/10.1016/j.clinph.2014.01.028

inion) to the mid frontal (Fz, as defined by the International 10–20 system) deviation with a 2–250 Hz band-pass filter. Rejection level was set to ±90 μV.

The stimulus conditions used were 3 reversals per second (rps) with small (8′) checks and 3 rps with large (65′) checks. Six hundred pattern-reversals (six blocks × 100 reversals, rejections not included) were delivered continuously for each stimulus condition. The order of check size presentation was randomized for each subject.

2.2.2. Navigated transcranial magnetic stimulation

The participants were scanned with a 3-T Siemens Trio MRI scanner. A T1 weighted 3D sequence was applied. Transcranial magnetic stimulation (TMS) was performed with a MagPro X100 unit with MagOption (Medtronic A/S, Tonsbakken 16–18, 2740 Skovlunde, Denmark) and an MCF-B65 Butterfly Coil (figure-of-eight magnetic coil) cooled with static fluid (MagVenture A/S, Lucernemarken 15, DK-3520 Farum, Denmark). Nexstim eXimia NBS version 2.2 (Nexstim Ltd, Elimäenkatu 9 B, FIN-00510 Helsinki Finland) was used for real-time navigation. Biphasic pulses with 280 μs duration were applied.

Resting motor threshold and phosphene threshold were measured to determine the rTMS output. Resting motor threshold was measured by stimulation of the primary motor cortex. Motor evoked responses were recorded with electromyography (EMG) on a Viking Select® system (Nicolet Biomedical Inc., Madison, WI USA). One-channel EMG responses were measured from the abductor pollicis brevis muscle of the right hand. The cathode electrode was placed on the muscle belly while the anode was placed on the first proximal phalanx. The patient-ground electrode was placed at the proximal end of the palmar surface of the hand.

The left gyrus precentralis was identified, and the 3D MRI model was pealed down until the gyrus was clearly visible. A mapping procedure was performed to locate the optimal coil position for stimulation. First, a coarse mapping of the gyrus precentralis was performed at 75% stimulator intensity and with 1 cm between stimulations, while the coil was held perpendicular to the longitudinal axis of the gyrus. This typically identified a 2–3 cm strip-area that included the omega-shaped (Ω) part associated with first order motor neurons controlling the hand muscles. This candidate-area was then mapped more thoroughly with one stimulus for every 1–2 mm. The stimulation point that evoked the highest peak-to-peak motor evoked potential amplitudes was chosen for further stimulation. Optimal coil rotation was determined by rotating the coil 22.5°, 45°, 67.5° and 90° clockwise and counter-clockwise. The stimulator output was thereafter reduced in steps of 5% until less than 5 out of 10 stimulations resulted in motor evoked potentials $\geqslant$50 μV. The output was then increased by 4% and reduced in steps of 1% until the resting motor threshold was found. The resting motor threshold was defined as the lowest output resulting in peak-to-peak motor evoked potentials $\geqslant$50 μV in at least 5 of 10 stimulations.

Phosphene thresholds were determined with a procedure similar to that of Kammer and Baumann (2010), who also used a Medtronic Magpro stimulator X100. Every 5×5 mm^2 of a 5×5 cm^2 grid over the occipital lobe was stimulated. The limits of the grid were 5 cm above the inion and 2.5 cm to each side of the inion. The lower limit was the inion or the border of the cerebellum. The primary visual cortex was always located within the grid. The stimulation took place in a dark room with the subject's eyes closed. Because prolonged light deprivation may increase cortical excitability in the occipital cortex (Boroojerdi et al., 2000), a break in the TMS was taken every 10–12 min. During these breaks the subjects' eyes were open and the lights were turned on. The coil was held with the tail upwards. A stimulator output of 70% was used. The subjects were told to report any visual phenomena observed in relation to the stimulation, and to rate its strength on an arbitrary scale from 1 to 10. Phosphene measurements depend on the subjective perception of phosphenes (Kammer et al., 2005). Therefore, an approved phosphene (to be included in further measurements and analysis) had to occur immediately after the stimulation, on the contralateral side of stimulation and be perceived both when the subject's eyes were closed and open. The stimulation point with the subjective highest rated strength was used to measure a phosphene threshold. If no valid phosphenes were observed, the same procedure was repeated at 100% stimulator output. The phosphene threshold was defined as the lowest stimulator output resulting in visible phosphenes in at least 3 out of 5 stimulations. This was determined by reducing the stimulator output in the same way as for resting motor thresholds.

2.2.3. High-frequency repetitive navigated transcranial magnetic stimulation

Navigated, high-frequency rTMS was performed at the location of phosphene threshold measurement or in the midline near the primary visual cortex (i.e. sulcus calcarinus) if no phosphenes were observed. Stimulus output during rTMS was equal to the phosphene threshold in subjects with phosphene thresholds below 76% of stimulator output. In accordance with previous studies (Bohotin et al., 2002; Fumal et al., 2006) stimulator output during rTMS was set to 110% of motor threshold in subjects with phosphene threshold above 75% of maximal stimulator output and in subjects not reporting phosphenes.

Each subject received 900 stimulations divided into 18 trains of 50 stimulations. The stimulation frequency was 10 Hz. The duration of each train was 5 s and there was a 10 s break between each train.

VEP examination was performed before rTMS, within 9 min after rTMS and 25 min after rTMS. Because the effects of rTMS on VEPs have been found to last for at least 9 min (Bohotin et al., 2002), the first VEP recordings after the rTMS were started within this time interval.

2.3. Data analysis and statistics

Analysis of VEP recordings was performed with Labchart 7.1.2 software (ADInstrument Pty Ltd, Sydney Australia). VEPs were averaged in 6 blocks of 100 averaged responses. An experienced neurophysiologist (TS) identified N70 (N1), P100 (P1) and N145 (N2) VEP peaks. The neurophysiologist was blinded to block number, subject diagnosis, and relation to rTMS. The neurophysiologist was not blinded to check size because VEP latency is evidently shorter with 65′ than 8′ checks. To ensure that the same VEP components were selected for all VEP recordings in one subject, the neurophysiologist also knew which blocks of responses belonged to the same subject.

Linear amplitude decrement over the six blocks (habituation slope) was used as primary habituation measure. Ratio between the first and last block (block ratio) was also calculated.

Measurements in CO, MINT and MPRE were compared. In addition, measurements in MwoA and MA were compared.

N70-P100 and P100-N145 VEP peak-to-peak amplitudes were square root transformed prior to analysis. The distributions of VEP amplitudes were skewed, and the transformation improved the distribution. Block ratios were log-transformed. For brainstem auditory evoked potentials it has been shown that square root transformations are best for amplitudes while ratios are best handled by a log-transformation (Sand, 1990). Habituation slopes were calculated with least squares linear regression for each subject. Block ratios, habituation slopes and first block amplitudes were

Please cite this article in press as: Omland PM et al. Modulation of visual evoked potentials by high-frequency repetitive transcranial magnetic stimulation in migraineurs. Clin Neurophysiol (2014), http://dx.doi.org/10.1016/j.clinph.2014.01.028

analysed with repeated measures ANOVA with group as between-subject factor and check size and rTMS (baseline, directly after rTMS and 25 min after rTMS) as within-subject factors. Huynh–Feldt correction was applied. Comparisons of VEP amplitude and habitation at baseline have been published earlier (Omland et al., 2013).

Phosphene prevalence was compared with the chi-square test, and Yates correction was applied. When comparing phosphene thresholds, thresholds were set to 101% in subjects who did not experience phosphenes. Phosphene thresholds and stimulator output applied during rTMS were compared with Mann–Whitney *U*-test.

A two sample Student's *t*-test comparing CO ($n = 32$) and MINT ($n = 25$) has 80% power to detect a medium population group difference equal to 0.75 SD. Two sample *t*-tests comparing MINT ($n = 25$) and MPRE ($n = 7$) have 80% power to detect a large difference equal to 1.2 SD. Two-sided *p*-values <0.05 are reported as significant in the primary two- and three-group comparisons. In post hoc analysis, a Bonferroni-type correction for three-group comparisons was applied and two-sided *p*-values <0.0167 are reported as significant. No further adjustments for multiple variables were applied because too extensive Bonferroni-type corrections are associated with increased number of type II errors (Perneger, 1998).

3. Results

Grand average VEPs elicited by small 8′ checks and large 65′ checks are shown in Figs. 1 and 2. For the comparison of CO, MINT and MPRE, significant interactions of group by check size and rTMS were found for the N70-P100 habituation measures (Table 2). Post hoc comparison of N70-P100 habituation slope for CO and MINT showed an interaction between rTMS and group for large checks (Table 3). With large checks, N70-P100 habituation slope indicated increased habituation in CO and reduced habituation in MINT both directly after rTMS and 25 min after rTMS (Figs. 3 and 4). With large checks, N70-P100 habituation also tended to be reduced in MPRE after rTMS, but this was not different from the effect in CO (Table 3 and Fig. 3).

Post hoc analysis of N70-P100 habituation measures for the CO and MPRE comparisons and the MINT and MPRE comparisons showed significant group by rTMS interactions for small checks (Table 3). With small checks, N70-P100 habituation measures indicated unchanged habituation in CO, increased habituation in MINT and reduced habituation in MPRE directly after rTMS (Fig. 3). Twenty-five minutes after rTMS the N70-P100 habitation measures approached the values observed before rTMS in all groups (Fig. 3).

Significant interactions between rTMS and group were found for P100-N145 habituation measures (Table 2). Post hoc analysis of P100-N145 habituation slope for the CO and MPRE comparisons and the MPRE and MINT comparisons showed significant interactions between rTMS and group for small checks (Table 3). P100-N145 habituation slopes with small checks indicated unchanged habituation in CO, increased habituation in MINT and reduced habituation in MPRE directly after rTMS (Figs. 3 and 5). P100-N145 habituation slopes approached the values observed before rTMS after 25 min (Fig. 3). With large checks, P100-N145 habituation also tended to be reduced in MPRE after rTMS, but this was not different from the effect in CO (Table 3 and Fig. 3).

Significant interactions of group by check size and rTMS were found for N70-P100 and P100-N145 first block amplitudes (Table 4). However, post hoc two-group comparisons for each check size showed no significant rTMS × group or rTMS effects ($p > 0.025$). The significant three-way interactions were likely related to a complex effect of check size and rTMS, probably reflecting that a general trend for amplitude reduction after 25 min was more pronounced with small checks in CO and with large checks in migraine.

No differences in the effect of rTMS on habituation slope or first block amplitude were found for the comparison of MwoA and MA

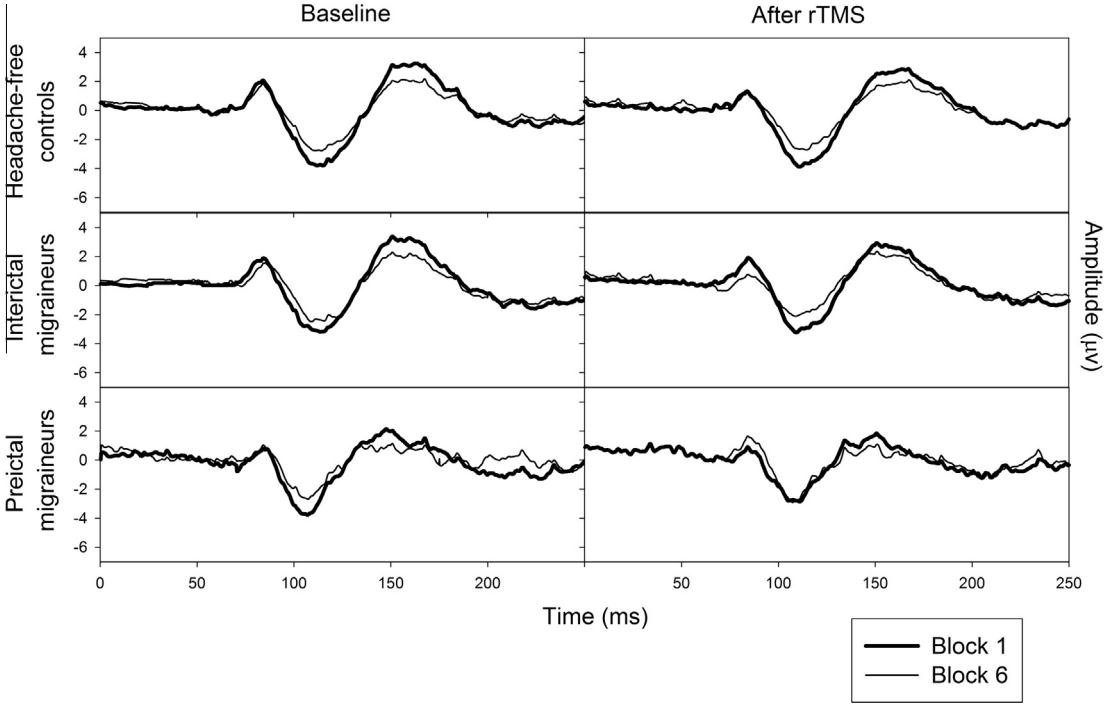

Fig. 1. Grand average VEPs elicited by small 8′ checks before and after high-frequency rTMS. Block 1 (thick lines) is the average of the first 100 responses (response 1–100) and block 6 (thin lines) is the average of the last 100 responses (response 501–600). In headache-free controls and interictal migraine VEP habituation was unchanged or increased after rTMS. In preictal migraine habituation was reduced after rTMS.

Please cite this article in press as: Omland PM et al. Modulation of visual evoked potentials by high-frequency repetitive transcranial magnetic stimulation in migraineurs. Clin Neurophysiol (2014), http://dx.doi.org/10.1016/j.clinph.2014.01.028

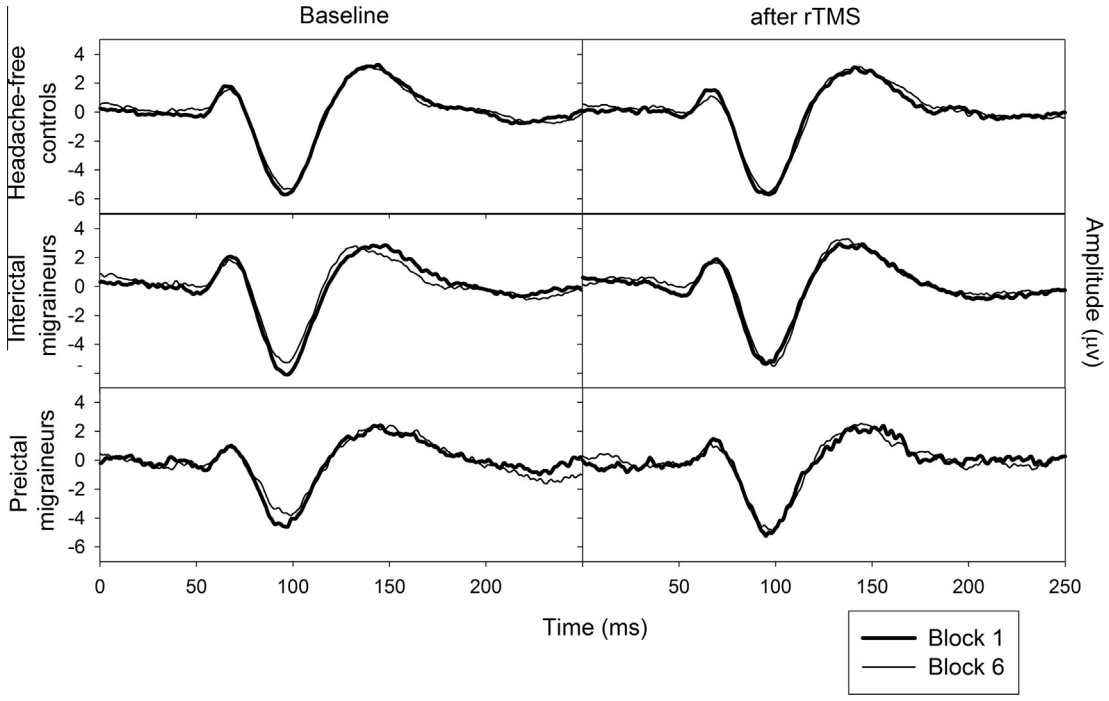

Fig. 2. Grand average VEPs elicited by large 65′ checks before and 25 min after high-frequency rTMS. Block 1 (thick lines) is the average of the first 100 responses (response 1–100) and block 6 (thin lines) is the average of the last 100 responses (response 501–600). VEP habituation increased in headache-free controls after rTMS, but decreased after rTMS in the migraine groups.

Table 2
Comparison of VEP habituation measures between CO, MINT and MPRE. Repeated measures ANOVA with habituation slope and block ratio as dependent variables, check size and rTMS (baseline, directly after rTMS and 25 min after rTMS) as within subject factors and group as between subject factor. *F*-Statistic values have been tabulated.

		Check size	Check size × group	rTMS	rTMS × group	Check size × rTMS	Check size × rTMS × group	Group
N70-P100	Habituation slope	13.0*	0.9	0.6	2.3	1.2	4.0*	0.1
	Block ratio	17.4*	0.2	3.2*	2.1	1.4	2.7*	0.7
P100-N145	Habituation slope	16.6*	0.07	0.6	2.7*	0.001	2.3	0.4
	Block ratio	24.4*	0.2	1.9	2.4*	0.4	2.0	0.7

Habituation slope: Least squares linear regression of block amplitudes. Block ratio: Block 6/block 1 amplitude ratio. VEP: Visual evoked potential. CO: Healthy controls. MINT: Interictal migraine. MPRE: Preictal migraine. rTMS: Repetitive transcranial magnetic stimulation. ×: interaction.
* $p < 0.05$.

($p > 0.20$). As reported previously, VEP amplitude and habituation were not different between CO and MINT before rTMS (Omland et al., 2013).

Phosphenes were found in 57.1% of MwoA and 81.8% of MA. Phosphene thresholds (mean ± SD) were 86.7% ± 16.3 in MwoA and 85.3% ± 15.0 in MA. Applied rTMS output was 56.6 ± 10.8% in MwoA and 60.3 ± 9.3% in MA. Phosphene prevalence, phosphene threshold and applied rTMS output were not significantly different in MwoA and MA ($p > 0.20$).

Phosphenes were found in 78.1% of CO, 68.0% of MINT and 85.7% of MPRE. Phosphene thresholds were 82.7% ± 16.6 in CO, 86.1% ± 15.4 in MINT and 82.1% ± 13.2% in MPRE. Applied rTMS output (mean ± SD) was 57.6% ± 10.5 in CO, 58.2% ± 10.1 in MINT and 58.6% ± 11.0 in the MPRE group. Phosphene prevalence, phosphene threshold or applied rTMS output were not significantly different in CO, MINT and MPRE ($p > 0.20$).

No serious adverse events (including seizures) occurred in this study.

4. Discussion

In this blinded case-control study, our main finding was that MINT, MPRE and CO responded differently to high-frequency rTMS. With large checks, rTMS reduced N70-P100 VEP habituation in MINT compared to CO. With small checks, rTMS reduced habituation in MPRE, while rTMS increased or had little effect on habituation in MINT and CO.

4.1. Comparisons with other studies

We observed increased VEP habituation with small checks in MINT after rTMS. This is similar to findings reported in an earlier study (Bohotin et al., 2002). The same study also reported that rTMS restored first block amplitudes and habituation to normal in MINT. This is in contrast to our findings as we only found differences between groups after rTMS. The authors of the former study concluded that rTMS increased an initially reduced cortical pre-activation level in the migraine group (Bohotin et al., 2002). Unfortunately, the results of earlier VEP studies are conflicting and do not provide a clear answer to how first block amplitude and habituation are altered in MINT. Therefore, these studies cannot prove the proposed and attractive concept of reduced cortical pre-activation in MINT (Omland et al., 2013). There are some differences between the study by Bohotin et al. (2002) and our study. The former study recruited outpatients, while the subjects in the present study were recruited from students and employees at

Table 3
Post hoc comparison for each check size of VEP habituation measures between CO, MINT and MPRE. Repeated measures ANOVA with habituation slope and block ratio as dependent variables and rTMS (baseline, directly after rTMS and 25 min after rTMS) as within subject factor and group as between subject factor. *F*-Statistics are tabulated.

		rTMS	rTMS × group	Group
N70-P100 VEP habituation				
CO-MINT (8′ checks)[1]	Habituation slope	2.5	1.1	0.2
	Block ratio	2.6	1.1	0.01
CO-MINT (65′ checks)[1]	Habituation slope	0.6	4.5*	1.1
	Block ratio	1.5	1.7	2.0
CO-MPRE (8′ checks)	Habituation slope	3.3	4.5*	0.04
	Block ratio	4.6*	5.5*	0.2
CO-MPRE (65′ checks)	Habituation slope	0.6	1.3	0.1
	Block ratio	0.2	0.2	0.1
MINT-MPRE (8′ checks)[1]	Habituation slope	1.0	5.7*	0.02
	Block ratio	2.1	6.0*	0.5
MINT-MPRE (65′ checks)[1]	Habituation slope	1.8	0.2	0.1
	Block ratio	2.4	0.1	0.4
P100-N145 VEP-habituation				
CO-MINT (8′ checks)	Habituation slope	5.6*	0.9	0.2
	Block ratio	6.2*	0.8	0.1
CO-MINT (65′ checks)	Habituation slope	0.05	1.2	2.2
	Block ratio	0.7	2.5	0.7
CO-MPRE (8′ checks)	Habituation slope	1.8	4.8*	0.1
	Block ratio	1.0	3.3	0.02
CO-MPRE (65′ checks)	Habituation slope	0.4	2.8	0.9
	Block ratio	0.8	1.4	0.2
MINT-MPRE (8′ checks)	Habituation slope	0.7	7.7*	0.1
	Block ratio	0.9	4.1	0.2
MINT-MPRE (65′ checks)	Habituation slope	0.9	0.2	0.6
	Block ratio	1.7	0.9	1.1

Habituation slope: Least squares linear regression of block amplitudes. Block ratio: Block 6/block 1 amplitude ratio. VEP: Visual evoked potential. CO: Healthy controls. MINT: Interictal migraine. MPRE: Preictal migraine. rTMS: Repetitive transcranial magnetic stimulation. ×: interaction.
[1] Post hoc ANOVAs.
* $p < 0.0167$.

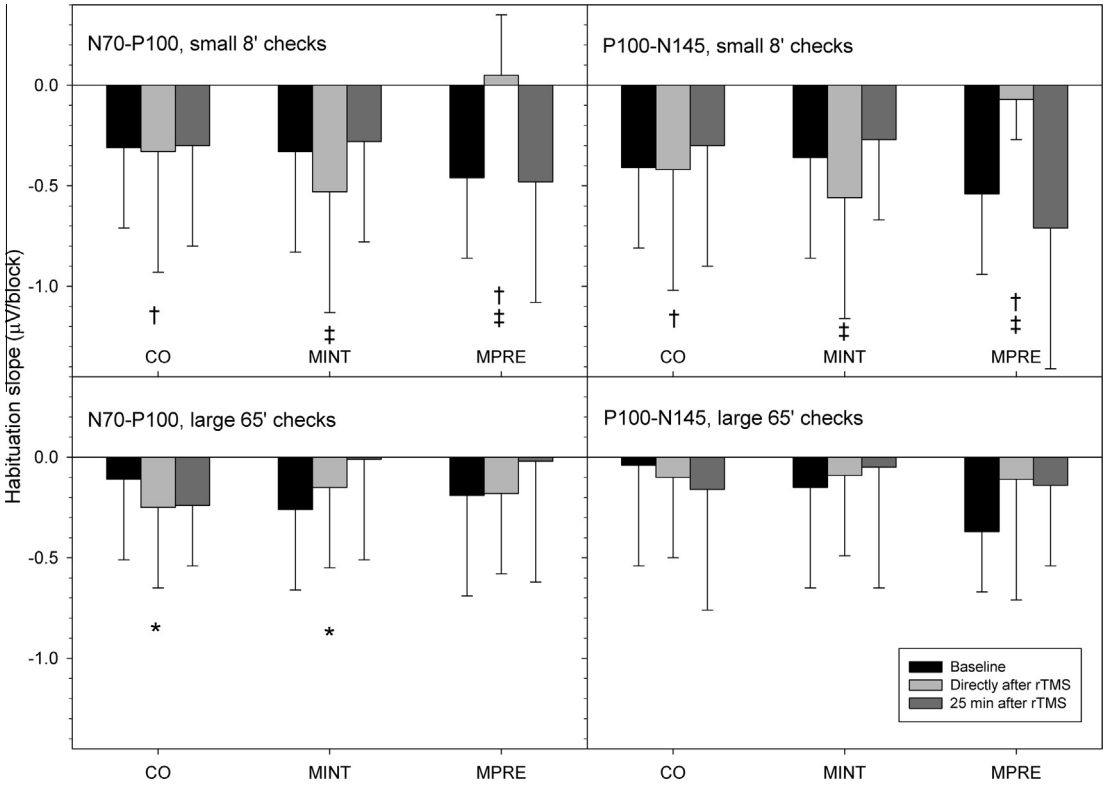

Fig. 3. VEP habituation slopes (mean ± SD, linear amplitude change over block calculated by the least squares method) before, directly after and 25 min after high-frequency rTMS. CO: Healthy controls; MINT: Migraineurs in the interictal period. MPRE: Migraineurs in the preictal period. *Significantly different effect of rTMS (baseline, directly after rTMS and 25 min after rTMS) on habituation slopes in CO and MINT (significant interaction of group by rTMS in repeated measures ANOVA). †Significantly different effect of rTMS on habituation slope in CO and MPRE (significant interaction of group by rTMS in repeated measures ANOVA). ‡Significantly different effect of rTMS on habituation slope in MINT and MPRE (significant interaction of group by rTMS in repeated measures ANOVA).

Please cite this article in press as: Omland PM et al. Modulation of visual evoked potentials by high-frequency repetitive transcranial magnetic stimulation in migraineurs. Clin Neurophysiol (2014), http://dx.doi.org/10.1016/j.clinph.2014.01.028

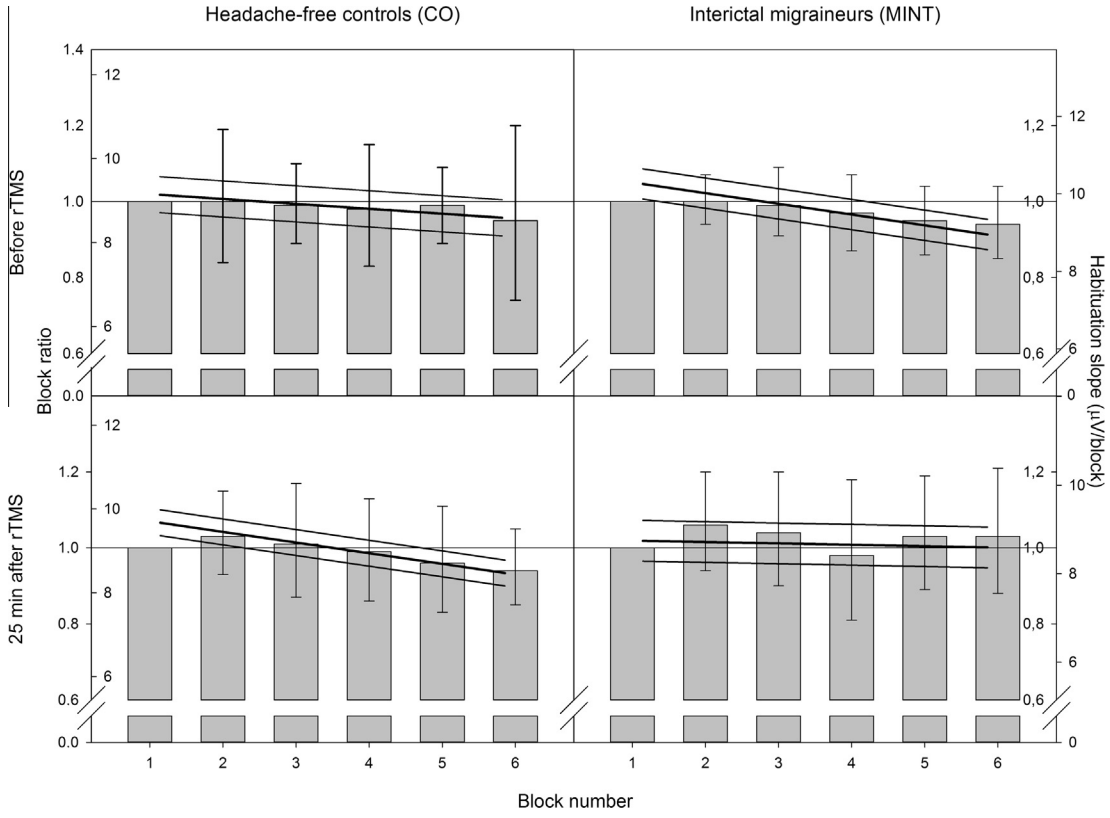

Fig. 4. N70-P100 amplitude habituation with large 65′ checks before (baseline recording) and 25 min after rTMS. Habituation slopes (straight lines, mean ± SD; μV/block) and block ratios (bars, block 1 mean = 1, mean ± SD) are shown. Habituation slopes were calculated from block amplitude with least squares linear regression. Block ratio is the ratio between the amplitude of block 2–6 and block 1. In this figure, habituation slopes were anchored between the block ratios of block 3 and 4. Habituation slopes and block ratios indicate reduced habituation in MINT after rTMS, but unchanged habituation in CO.

our university. Still, the reported headache frequency and disease duration in these two studies were similar. Only the present study recruited controls with similar age as the migraineurs.

We found differences between CO and MINT with large checks, but not small checks. This is in agreement with earlier findings indicating that the magnocellular pathway is affected in MINT (Chen et al., 2009). Thus, changes in visual responses may be easier to detect with certain stimulation parameters, and may not be generalized as some authors have suggested (Coppola et al., 2013). RTMS reduced VEP habituation with small checks in MPRE compared to MINT and CO. Therefore the parvocellular pathway may be affected in the preictal period. We found the same tendencies for MPRE with large checks. However, these findings were not significant, possibly because of the rather few MPRE subjects. Increased amplitude in VEPs with large checks has been found in MPRE (Sand et al., 2009), indicating that the magnocellular pathway also is affected.

Phosphene thresholds were not different in MINT and CO before rTMS in the present study. This finding is in accordance with a meta-analysis of phosphene threshold and prevalence in CO, MwoA and MA: No differences in phosphene threshold or prevalence were found in studies using figure-of-eight TMS coils (Brigo et al., 2013).

4.2. Interpretation

We did not find differences in VEPs (Omland et al., 2013) or phosphenes between groups before rTMS. Our results do therefore not support the notion that the migraine cortex is simply characterized by altered cortical excitability. Habituation remained stable or increased in CO after rTMS, while the migraineurs showed an increased responsivity to rTMS. Subtle differences in the visual cortices of migraineurs and CO therefore probably exist, but we were unable to detect these with our baseline measures. Interestingly, MA may have a dysfunction in inhibitory regulative mechanisms of the motor cortex, resulting in a reduced ability to prevent excessive increases in cortical excitability (Antal et al., 2008). We hypothesize that a similar dysfunction in the visual cortex may have caused the relative lack of habituation after rTMS in the migraine groups in the present study.

The underlying mechanisms for the differential involvement of the magnocellular and parvocellular system in migraine are not known. Still, abnormalities of the magnocellular system could explain why we found different effects of rTMS on VEP in MINT and CO. Several studies have indicated that migraineurs have deficits in the processing of visual motion (Antal et al., 2005, 2011; Battista et al., 2010, 2011; McKendrick and Badcock, 2004; Shepherd, 2006; Shepherd et al., 2012). These deficits are likely to represent abnormalities of the magnocellular rather than the parvocellular visual subsystem (McKendrick et al., 2001). In addition, studies have showed increased excitability (Battelli et al., 2002) and thickness abnormalities of the cortical V3A area (Granziera et al., 2006), which is sensitive to motion (Tootell et al., 1997). This area is also close to the possible source of cortical spreading depression, which is believed to cause the migraine aura (Hadjikhani et al., 2001).

Although the effect of rTMS was significantly different in MPRE and CO only when small and not when large checks were applied, the findings in MPRE may indicate that the cortical dysfunction increases close to an attack. An alternative explanation is that only the parvocellular system is affected preictally, but a dysfunction shifting from the magnocellular to the parvocellular subsystem

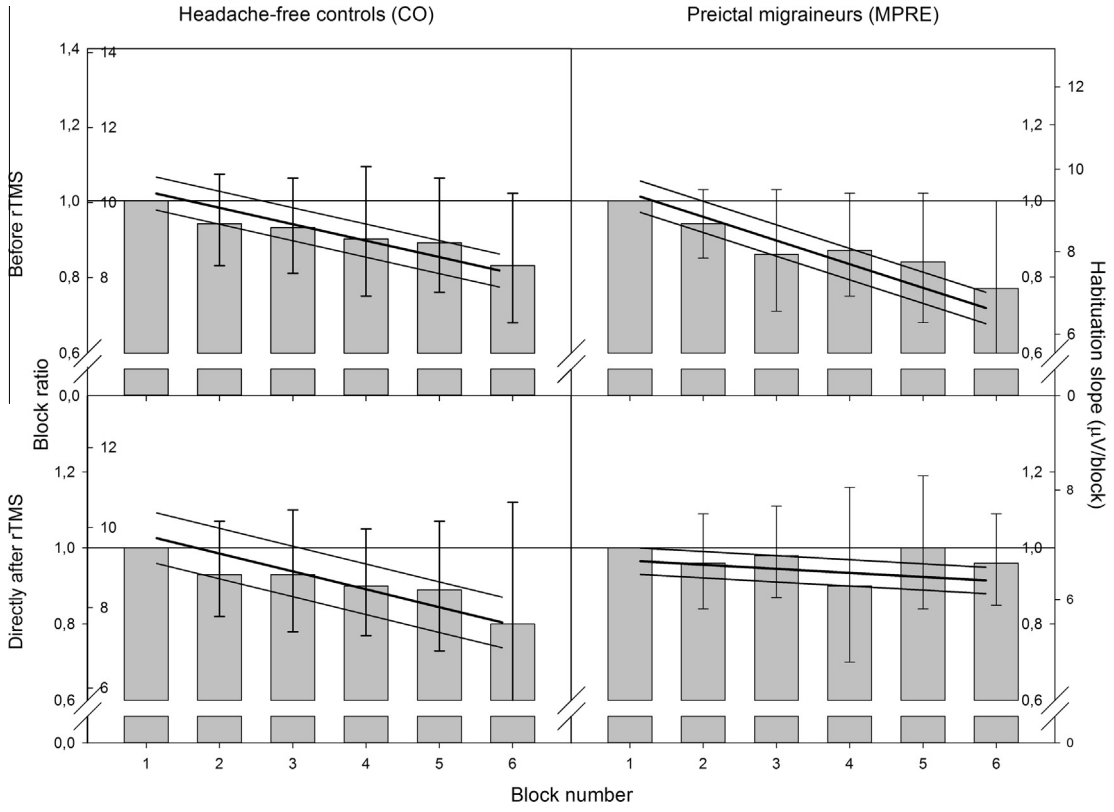

Fig. 5. P100-N145 amplitude habituation with small 8′ checks before (baseline recording) and 25 min after rTMS. Habituation slopes (straight lines, mean ± SD; µV/block) and block ratios (bars, block 1 mean = 1, mean ± SD) are shown. Habituation slopes were calculated from block amplitude with least squares linear regression. Block ratio is the ratio between the amplitude of block 2–6 and block 1. In this figure, habituation slopes were anchored between the block ratios of block 3 and 4. Habituation slopes and block ratios indicate reduced habituation in MPRE compared to CO.

Table 4
Comparison of first block VEP amplitudes between CO, MINT and MPRE. Repeated measures ANOVA with first block amplitude as dependent variable, check size and rTMS (baseline, directly after rTMS and 25 min after rTMS) as within subject factors, and group as between subject factor. *F*-Statistic values have been tabulated.

	Check size	Check size × group	rTMS	rTMS × group	Check size × rTMS	Check size × rTMS × group	Group
N70-P100	5.2*	0.6	5.7*	1.6	0.2	3.1*	0.1
P100-N145	13.1*	0.7	4.2*	1.9	1.1	2.6*	0.4

VEP: Visual evoked potential. CO: Healthy controls. MINT: Interictal migraine. MPRE: Preictal migraine. rTMS: Repetitive transcranial magnetic stimulation. ×: Interaction.
* $p < 0.05$.

in the preictal period does not seem plausible. An increased cortical dysfunction preictally is also in general agreement with longitudinal measurements of QEEG, which found indications of reduced inhibitory mechanisms in the cortex of MPRE (Bjork et al., 2011). On the other hand, pre- and peri-ictal normalisation of visual responses have also been reported (Chen et al., 2009; Judit et al., 2000). The diverging results of the different studies are not easy to interpret. Looking ahead of the divergence, these preictal changes are generally consistent with the concept that a migraine attack starts in the central nervous system. The diversity of the results also suggests that the traits of migraine are multifarious. In addition, results may depend on methodological details.

We could not find support for a reduced cortical pre-activation level in migraine. However, this theory has also been supported by studies that used different methods such as QEEG (Bjork et al., 2011) and somatosensory evoked potentials (Coppola et al., 2005). Hence, first block VEP amplitude may not be a sufficiently sensitive and reliable measure of cortical excitability. For instance it is not known if the "first block" should be defined as the average of 10, 20, 50 or 100 reversals. Differences within the first 10–20 responses are difficult to detect because of the low signal/noise ratio. Such transient changes may not have been detected with the standard VEP protocol applied in the present study. Other study protocols may be better suited to investigate transient effects (i.e. Hoffken et al., 2009).

The effect of rTMS may depend on the underlying cortical excitability (Fierro et al., 2005). High-frequency rTMS over the motor cortex has shown unexpected paradoxical effects in migraineurs in that stimulation with 110% stimulator output resulted in facilitation of motor evoked potentials, while inhibition was seen when 130% stimulator output was applied (Brighina et al., 2011). Thus, the findings by Bohotin et al. (2002) may have an alternative interpretation. The normalisation of VEPs after rTMS may have been caused by high-frequency rTMS *reducing* the excitability of a primarily hyperexcitable cortex (Brighina et al., 2009). However, the reduction in habituation in our migraine groups after rTMS suggests *reduced cortical inhibition* as a more likely explanation. In addition, the paradoxical effect of rTMS in migraine was reported with a stimulator output that probably was higher than the output used in the present study (Brighina et al., 2011).

The effect of rTMS on P100-N145 habituation in CO and MINT were similar to the effect on N70-P100 habituation, but it was

not significant. The effect was also not significant for block ratio. However, many VEP studies have only analysed the N70-P100 component (see Table 5 in Omland et al., 2013) and habituation slope is probably a better measure of habituation than block ratio (Omland et al., 2011). Still, our findings would have been more robust if they were significant for the P100-N145 amplitude and block ratio as well.

4.3. Strengths and limitations

The present study was blinded during recoding and analysis of VEPs, ensuring unbiased evaluation of the effect of rTMS. In addition, the applied stimulator intensities during rTMS were similar in the different groups. It is therefore unlikely that differences related to the procedure could be causing biases. A limitation in the present study is the small MPRE group. Because we did not find any differences in the effect of rTMS on VEPs in MA and MwoA, we chose to merge the MwoA and MA groups. Earlier studies have not found differences in VEP habitation between MwoA and MA (Afra et al., 1998, 2000b; Coppola et al., 2007a; Ozkul and Bozlar, 2002; Schoenen et al., 1995). However, it can be argued that MwoA and MA should be separated both in the interictal and preictal groups if the power of the study is sufficient. For instance, glutamatergic transmission may differ in patients with and without aura (Conte et al., 2010). Our results should therefore be validated, preferably in a longitudinal study with larger subgroups.

The duration of the effect of high-frequency rTMS is likely short (Bohotin et al., 2002). This limited the number of variables that could be measured after rTMS. We therefore did not measure phosphene thresholds after rTMS.

5. Conclusion

The migraine visual cortex showed an increased responsivity to high-frequency rTMS, causing a relative lack of habituation compared to controls. This dysfunction seems to change in the preictal period, which may be a sign of a developing migraine attack. The magnocellular subsystem may be affected in MINT. In MPRE the parvocellular subsystem may be affected as well. We could not confirm that migraineurs have an altered cortical pre-activation level that can be restored by high-frequency rTMS.

6. Funding

This study received funding from the Norwegian University of Science and Technology and the Royal Norwegian Society of Sciences and Letters.

Acknowledgement

We are most grateful for invaluable assistance from Marit Stjern and Gøril Gravdahl.

These authors declare no conflict of interest.

References

Afra J, Cecchini AP, De Pasqua V, Albert A, Schoenen J. Visual evoked potentials during long periods of pattern-reversal stimulation in migraine. Brain 1998;121:233–41.

Afra J, Ambrosini A, Genicot R, Albert A, Schoenen J. Influence of colors on habituation of visual evoked potentials in patients with migraine with aura and in healthy volunteers. Headache 2000a;40:36–40.

Afra J, Proietti Cecchini A, Sandor PS, Schoenen J. Comparison of visual and auditory evoked cortical potentials in migraine patients between attacks. Clin Neurophysiol 2000b;111:1124–9.

Antal A, Temme J, Nitsche MA, Varga ET, Lang N, Paulus W. Altered motion perception in migraineurs: evidence for interictal cortical hyperexcitability. Cephalalgia 2005;25:788–94.

Antal A, Lang N, Boros K, Nitsche M, Siebner HR, Paulus W. Homeostatic metaplasticity of the motor cortex is altered during headache-free intervals in migraine with aura. Cereb Cortex 2008;18:2701–5.

Antal A, Polania R, Saller K, Morawetz C, Schmidt-Samoa C, Baudewig J, et al. Differential activation of the middle-temporal complex to visual stimulation in migraineurs. Cephalalgia 2011;31:338–45.

Aurora SK, Wilkinson F. The brain is hyperexcitable in migraine. Cephalalgia 2007;27:1442–53.

Bassi CJ, Lehmkuhle S. Clinical implications of parallel visual pathways. J Am Optom Assoc 1990;61:98–110.

Battelli L, Black KR, Wray SH. Transcranial magnetic stimulation of visual area V5 in migraine. Neurology 2002;58:1066–9.

Battista J, Badcock DR, McKendrick AM. Center-surround visual motion processing in migraine. Invest Ophthalmol Vis Sci 2010;51:6070–6.

Battista J, Badcock DR, McKendrick AM. Migraine increases centre-surround suppression for drifting visual stimuli. PLoS One 2011;6:e18211.

Bjork M, Stovner LJ, Hagen K, Sand T. What initiates a migraine attack? Conclusions from four longitudinal studies of quantitative EEG and steady-state visual-evoked potentials in migraineurs. Acta Neurol Scand Suppl 2011:56–63.

Bohotin V, Fumal A, Vandenheede M, Gerard P, Bohotin C, Maertens de Noordhout A, et al. Effects of repetitive transcranial magnetic stimulation on visual evoked potentials in migraine. Brain 2002;125:912–22.

Boroojerdi B, Bushara KO, Corwell B, Immisch I, Battaglia F, Muellbacher W, et al. Enhanced excitability of the human visual cortex induced by short-term light deprivation. Cereb Cortex 2000;10:529–34.

Brighina F, Palermo A, Fierro B. Cortical inhibition and habituation to evoked potentials: relevance for pathophysiology of migraine. J Headache Pain 2009;10:77–84.

Brighina F, Cosentino G, Vigneri S, Talamanca S, Palermo A, Giglia G, et al. Abnormal facilitatory mechanisms in motor cortex of migraine with aura. Eur J Pain 2011;15:928–35.

Brigo F, Storti M, Tezzon F, Manganotti P, Nardone R. Primary visual cortex excitability in migraine: a systematic review with meta-analysis. Neurol Sci 2013;34:819–30.

Chen WT, Wang SJ, Fuh JL, Lin CP, Ko YC, Lin YY. Peri-ictal normalization of visual cortex excitability in migraine: an MEG study. Cephalalgia 2009;29:1202–11.

Conte A, Barbanti P, Frasca V, Iacovelli E, Gabriele M, Giacomelli E, et al. Differences in short-term primary motor cortex synaptic potentiation as assessed by repetitive transcranial magnetic stimulation in migraine patients with and without aura. Pain 2010;148:43–8.

Coppola G, Vandenheede M, Di Clemente L, Ambrosini A, Fumal A, De Pasqua V, et al. Somatosensory evoked high-frequency oscillations reflecting thalamo-cortical activity are decreased in migraine patients between attacks. Brain 2005;128:98–103.

Coppola G, Ambrosini A, Di Clemente L, Magis D, Fumal A, Gerard P, et al. Interictal abnormalities of gamma band activity in visual evoked responses in migraine: an indication of thalamocortical dysrhythmia? Cephalalgia 2007a;27:1360–7.

Coppola G, Pierelli F, Schoenen J. Is the cerebral cortex hyperexcitable or hyperresponsive in migraine? Cephalalgia 2007b;27:1427–39.

Coppola G, Pierelli F, Schoenen J. Habituation and migraine. Neurobiol Learn Mem 2009;92:249–59.

Coppola G, Di Lorenzo C, Schoenen J, Pierelli F. Habituation and sensitization in primary headaches. J Headache Pain 2013;14:65.

Fierro B, Brighina F, Vitello G, Piazza A, Scalia S, Giglia G, et al. Modulatory effects of low- and high-frequency repetitive transcranial magnetic stimulation on visual cortex of healthy subjects undergoing light deprivation. J Physiol 2005;565:659–65.

Fumal A, Coppola G, Bohotin V, Gerardy PY, Seidel L, Donneau AF, et al. Induction of long-lasting changes of visual cortex excitability by five daily sessions of repetitive transcranial magnetic stimulation (rTMS) in healthy volunteers and migraine patients. Cephalalgia 2006;26:143–9.

Giffin NJ, Ruggiero L, Lipton RB, Silberstein SD, Tvedskov JF, Olesen J, et al. Premonitory symptoms in migraine: an electronic diary study. Neurology 2003;60:935–40.

Granziera C, DaSilva AF, Snyder J, Tuch DS, Hadjikhani N. Anatomical alterations of the visual motion processing network in migraine with and without aura. PLoS Med 2006;3:e402.

Hadjikhani N, Sanchez Del Rio M, Wu O, Schwartz D, Bakker D, Fischl B, et al. Mechanisms of migraine aura revealed by functional MRI in human visual cortex. Proc Natl Acad Sci USA 2001;98:4687–92.

Hansen JM, Bolla M, Magis D, de Pasqua V, Ashina M, Thomsen LL, et al. Habituation of evoked responses is greater in patients with familial hemiplegic migraine than in controls: a contrast with the common forms of migraine. Eur J Neurol 2011;18:478–85.

Headache Classification Committee of the International Headache Society. The International Classification of Headache Disorders, 2nd ed.. Cephalalgia 2004;24(Suppl. 1):9–160.

Hoffken O, Stude P, Lenz M, Bach M, Dinse HR, Tegenthoff M. Visual paired-pulse stimulation reveals enhanced visual cortex excitability in migraineurs. Eur J Neurosci 2009;30:714–20.

Judit A, Sandor PS, Schoenen J. Habituation of visual and intensity dependence of auditory evoked cortical potentials tends to normalize just before and during the migraine attack. Cephalalgia 2000;20:714–9.

Kammer T, Puls K, Erb M, Grodd W. Transcranial magnetic stimulation in the visual system II. Characterization of induced phosphenes and scotomas. Exp Brain Res 2005;160:129–40.

Please cite this article in press as: Omland PM et al. Modulation of visual evoked potentials by high-frequency repetitive transcranial magnetic stimulation in migraineurs. Clin Neurophysiol (2014), http://dx.doi.org/10.1016/j.clinph.2014.01.028

Kammer T, Baumann LW. Phosphene thresholds evoked with single and double TMS pulses. Clin Neurophysiol 2010;121:376–9.
Kandel E, Schwartz J, Jessell T. Principles of Neural Science. 5th ed. New-York: McGraw-Hill Education; 2013.
Khalil NM, Legg NJ, Anderson DJ. Long term decline of P100 amplitude in migraine with aura. J Neurol Neurosurg Psychiatry 2000;69:507–11.
Livingstone M, Hubel D. Segregation of form, color, movement, and depth: anatomy, physiology, and perception. Science 1988;240:740–9.
McKendrick AM, Vingrys AJ, Badcock DR, Heywood JT. Visual dysfunction between migraine events. Invest Ophthalmol Vis Sci 2001;42:626–33.
McKendrick AM, Badcock DR. Motion processing deficits in migraine. Cephalalgia 2004;24:363–72.
Noseda R, Burstein R. Migraine pathophysiology: anatomy of the trigeminovascular pathway and associated neurological symptoms, cortical spreading depression, sensitization, and modulation of pain. Pain 2013. http://dx.doi.org/10.1016/j.pain.2013.07.021.
Oelkers-Ax R, Parzer P, Resch F, Weisbrod M. Maturation of early visual processing investigated by a pattern-reversal habituation paradigm is altered in migraine. Cephalalgia 2005;25:280–9.
Oelkers R, Grosser K, Lang E, Geisslinger G, Kobal G, Brune K, et al. Visual evoked potentials in migraine patients: alterations depend on pattern spatial frequency. Brain 1999;122:1147–55.
Omland PM, Nilsen KB, Sand T. Habituation measured by pattern reversal visual evoked potentials depends more on check size than reversal rate. Clin Neurophysiol 2011;122:1846–53.
Omland PM, Nilsen KB, Uglem M, Gravdahl G, Linde M, Hagen K, et al. Visual evoked potentials in interictal migraine: no confirmation of abnormal habituation. Headache 2013;53:1071–86.
Ozkul Y, Bozlar S. Effects of fluoxetine on habituation of pattern reversal visually evoked potentials in migraine prophylaxis. Headache 2002;42:582–7.
Pascual-Leone A, Valls-Sole J, Wassermann EM, Hallett M. Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. Brain 1994;117:847–58.
Perneger TV. What's wrong with Bonferroni adjustments. BMJ 1998;316:1236–8.
Rankin CH, Abrams T, Barry RJ, Bhatnagar S, Clayton DF, Colombo J, et al. Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. Neurobiol Learn Mem 2009;92:135–8.
Sand T. Statistical properties of ABR amplitudes and latencies. Implications for computation of reference limits and relation to click phase. Scand Audiol 1990;19:131–7.
Sand T, Zhitniy N, White LR, Stovner LJ. Visual evoked potential latency, amplitude and habituation in migraine: a longitudinal study. Clin Neurophysiol 2008;119:1020–7.
Sand T, White LR, Hagen K, Stovner LJ. Visual evoked potential and spatial frequency in migraine: a longitudinal study. Acta Neurol Scand Suppl 2009:33–7.
Schoenen J, Wang W, Albert A, Delawaide PJ. Potentiation instead of habituation characterizes visual evoked potentials in migraine patients between attacks. Eur J Neurol 1995;2:115–22.
Shepherd AJ. Local and global motion after-effects are both enhanced in migraine, and the underlying mechanisms differ across cortical areas. Brain 2006;129:1833–43.
Shepherd AJ, Beaumont HM, Hine TJ. Motion processing deficits in migraine are related to contrast sensitivity. Cephalalgia 2012;32:554–70.
Tootell RB, Mendola JD, Hadjikhani NK, Ledden PJ, Liu AK, Reppas JB, et al. Functional analysis of V3A and related areas in human visual cortex. J Neurosci 1997;17:7060–78.
Vecchia D, Pietrobon D. Migraine: a disorder of brain excitatory-inhibitory balance? Trends Neurosci 2012;35:507–20.

Please cite this article in press as: Omland PM et al. Modulation of visual evoked potentials by high-frequency repetitive transcranial magnetic stimulation in migraineurs. Clin Neurophysiol (2014), http://dx.doi.org/10.1016/j.clinph.2014.01.028