Abnormal Changes of Synaptic Excitability in Migraine with Aura

Michael Siniatchkin,1,2 Mascha Sendacki,1 Friederike Moeller,1 Stephan Wolff,2 Olav Jansen,3 Hartwig Siebner4 and Ulrich Stephani1

1Neuropediatric Department, Christian Albrecht University, D-24105 Kiel, Germany, 2Clinic for Child and Adolescent Psychiatry, Psychosomatic and Psychotherapy, Philipp University of Marburg, D-35043 Marburg, Germany, 3Department of Neuroradiology, Christian-Albrechts-University, D-24105 Kiel, Germany and 4Danish Research Centre for Magnetic Resonance, Copenhagen University Hospital Hvidovre, Copenhagen, Denmark

Migraine patients are characterized by altered cortical excitability and information processing between attacks. The relationship between these abnormalities is still poorly understood. In this study, visual evoked potentials (VEP) and proton magnetic resonance spectroscopy were recorded simultaneously in migraineurs and healthy subjects. In order to investigate the homeostatic-like plasticity in the visual cortex, cortical excitability was modified using transcranial direct current stimulation (tDCS). Before any stimulation, migraineurs showed significantly higher glutamate/creatine ratios (Glx/Cr) than healthy subjects. In healthy subjects, excitatory (anodal) tDCS caused an increase and inhibitory (cathodal) tDCS a decrease in the Glx/Cr ratio. Subsequent photic stimulation (PS) reversed the changes in Glx/Cr ratios, which returned back to baseline, demonstrating homeostatic-like meta-plasticity in the control group. In migraine patients, both anodal and cathodal tDCS decreased the Glx/Cr ratio, which did not return to baseline after PS. While healthy subjects showed an increase in VEP amplitude under anodal and a reduction under cathodal tDCS, the modifiability of VEP under tDCS was reduced in migraineurs. The results demonstrate a reduced responsiveness of the occipital cortex to interventions that change cortical excitability in migraine. Moreover, altered glutamatergic neurotransmission seems to mediate the relation between abnormal cortical information processing and excitability in migraineurs.

Keywords: cortical excitability, homeostatic-like plasticity, migraine, transcranial direct current stimulation, visual cortex

Introduction

Migraine is associated with abnormal responsiveness of the brain to environmental stimuli (Coppola et al. 2009). Increased amplitudes and reduced habituation of evoked and event-related potentials are well-replicated findings in migraine (Afra, Cecchini et al. 1998; Siniatchkin, Kirsch, et al. 2000; Di Clemente et al. 2005; Sand et al. 2009). However, there is no consistent physiological explanation for this phenomenon. One line of research has provided evidence for reduced cortical excitability in migraine (Maertens de Noordhout et al. 1992; Van der Kamp et al. 1996; Afra, Mascia, et al. 1998; Bohotin et al. 2002). They claimed abnormal habituation to be a protective mechanism, which optimizes the cortical preactivation level (Bohotin et al. 2002). Others have found evidence for increased cortical excitability in migraine (Aurora et al. 1998; Gerwig et al. 2005; Gunaydin et al. 2006; Khedr et al. 2006; Siniatchkin et al. 2009). Here abnormal habituation and excessive excitability are conceptualized as 2 features of a common pathogenic mechanism, such as destabilization of the membrane potential following a dysfunction of neuronal ion channels (van den Maagdenberg et al. 2007).

The consequences of abnormal cortical reactivity in migraine are also unclear. It has been hypothesized that excessive cortical activation due to deficient habituation during repetitive stimulation as well as decreased brain mitochondrial energy reserves may cause biochemical shifts, which lead to migraine attacks via cortical spreading depression (CSD) (Schoenen 2006). Indeed, several studies with magnetic resonance spectroscopy (MRS) and visual stimulation have revealed increased lactate and decreased N-acetylaspartate (NAA) signals under photic stimulation (PS) in patients suffering from migraine with aura (Sandor et al. 2005; Sarchielli et al. 2005). However, in those studies, the induced brain metabolic activity and evoked neuronal activity were not measured simultaneously and the glutamate/glutamine (Glx) signals, which are included in the H1-magnetic resonance (MR) spectrum, were not considered. The tight relationship between glutamate, cortical excitability, and evoked neuronal activity and their important role in the pathogenesis of migraine (see for review, Ramadan 2003; Vikelis and Mitsikostas 2007) motivates studies that simultaneously assess changes in glutamate, cortical excitability, and information processing to obtain new insights into the pathogenesis of migraine.

Here, we report on simultaneous recordings of visual evoked potentials (VEP) and H1-MRS at 3-T in patients with migraine with aura. Since the sensitivity of Glx and NAA measurements increases with magnetic field strength (Lange et al. 2006), we studied Glx and NAA changes induced by intermittent PS and transcranial direct current stimulation (tDCS). Among other neurotransmitters mediating effects of both PS and tDCS on cortical excitability, glutamate seems to be one of the key mediators (Liebetanz et al. 2002; Nitsche et al. 2003; Korostenskaja et al. 2007; Stagg et al. 2009; Watson et al. 2009). Therefore, we reasoned that combining PS and tDCS should open an avenue for the investigation of mechanisms of adaptive synaptic plasticity in migraine.

Materials and Methods

Participants

Ten right-handed adolescents and young adults suffering from migraine with visual aura (6 women, mean age 19.3 ± 3.4 years) were recruited from the departmental database and matched with 10 healthy, age- and gender-matched volunteers (6 women, mean age 20.3 ± 3.2 years) (medical students). Structured headache interviews were performed with all participants, and the diagnosis of migraine was made by an experienced neurologist according to the revised criteria of the International Headache Society (IHS 2004, the International Classification of Headache Disorders, 2004, code 1.2.1).
Additionally, a prospective daily headache diary was used to assess headache characteristics over an 8-week period before the patients were included in the study (Pothmann 1993). The diagnosis of migraine with aura was validated by the headache diary in all patients. All migraine patients experienced visual disturbances during the aura phase consisting of positive and negative scotomata, flashes, and fortification spectra. Five of them also reported sensory disturbances in the same phase consisting of paresthesia in the hand, arm, and/or perioral zone ipsilaterally to the visual disturbances. Motor deficit symptoms were reported by 3 patients. The duration of auras never exceeded 60 min. In all patients, the frequency of migraine attacks varied between 0.1 and 2.3 per month (mean ± standard deviation [SD]: 1.1 ± 0.9 attacks per month), and attacks lasted for 8-36 h (mean ± SD: 21.8 ± 9.4 h). Patients suffered from migraine for 2-9 years (mean ± SD: 4.2 ± 1.1 years). Neurological and routine medical examinations revealed no additional health problems (including acute infection) other than primary headaches in all participants. In particular, none of the subjects presented with any psychiatric disorder, which would have fulfilled the diagnostic criteria of DSM-IV-TR (fourth edition, American Psychiatric Association, Washington, DC, 2000). None of the participants had used any prophylactic medication nor taken part in any nonpharmacological treatment program for at least 6 months prior to the investigation. None of the patients took acute antimigraine medication more than 2 times a month. Experiments were conducted during the headache-free interval. Care was taken that at least 5 days had elapsed between the attacks and the recording since in former studies evoked and event-related potentials showed periodic changes associated with the migraine interval (Sniatchkin et al. 1999; Sniatchkin, Gerber, et al. 2000; Sniatchkin, Kropp, et al. 2000). On the day of recording, all participants were well rested (at least 7 h of sleep). None of the subjects had had impairment or had drunk alcohol in the 3 days before investigation. Visual acuity was normal or corrected-to-normal as assessed by a Snellen chart. No recording was performed in the premenstrual period of the cycle of female subjects. All recordings were carried out in the afternoon.

The study was conducted with the permission of the Ethic Committee of the Faculty of Medicine, University of Kiel, Germany. All participants and their parents were informed in detail about the experimental procedure, and written informed consent according to the Declaration of Helsinki (current version, 1996) on biomedical research involving human subjects (Tokyo amendment) was obtained for all participants. All subjects were highly motivated and received a small cash compensation for their participation.

Experimenental Procedure

All participants were included in 2 experimental sessions: In one session, participants received anodal tDCS to the visual cortex and in the other session cathodal tDCS. The order of experimental sessions was counterbalanced across subjects with an interval of at least 7 days between sessions. The time line of measurements and interventions was identical for each session. Every single experimental session lasted approximately 40-45 min. The experiments were carried out by a technician with long-lasting experience in simultaneous electroencephalography–functional magnetic resonance imaging (EEG–fMRI) recordings who was unaware of the diagnosis and polarity of stimulation and able to set the electrode cap within a few minutes. Therefore, both patients and technician were unaware of the experimental condition. After setting the electrode cap, the participants were positioned in the MR scanner and the 2 preinterventional MRS measurements were performed. We first acquired MRS in darkness without PS (preinterventional baseline) followed by an MRS measurement during PS. We then applied either anodal or cathodal tDCS outside the scanner, depending on the experimental session. For tDCS, the electrode cap was completely removed (several seconds) and then replaced on the head. A second set of MRS measurements started ~5 min after the end of the tDCS session using an identical procedure as for preinterventional MRS. Hence, MRS was first performed without PS (postinterventional baseline) and then with PS.

Photic Stimulation

PS was performed during MRS using the same procedure as described in Moeller et al. (2009). PS consisted of unpatterned and achromatic flashes of light applied in a completely dark room. The subjects were asked to keep their eyes closed during the entire functional measurement. The light flashes were produced by a xenon-discharge Grass PS22 stimulator (Astro-Med, Inc.) located outside the scanner suite. Two 2 fiber optic cables (2 cm diameter each), the flashes of light were conducted into the scanner room and were presented to the subjects through goggles (Fig. 1). The frequency of stimulation was 2 Hz. Since PS lasted for 6 min (300 s), a total of 600 VEP trials were recorded during a single block of PS. The PS was strong enough to elicit a highly significant positive blood oxygenation level-dependent (BOLD) response in the occipital cortex as tested in 20 subjects with photosensitivity with fMRI (Fig. 1, see also Moeller et al. 2009). The fMRI activation maps that we had obtained in this previous study were used to select the voxel of interest (VOI) for MRS. The voxel was centered on the regional peak of PS-induced BOLD activation in the group activation map (Moeller et al. 2009).

Visual Evoked Potentials

The EEG was continuously recorded inside the magnetic resonance imaging (MRI) scanner from 30 scalp sites (10–20 system plus FC1, FC2, CP1, PC2, FC5, FC6, CP5, CP6, T9, T10, TP10) with a reference located between Fz and Cz. Sintered Ag/AgCl ring electrodes were attached using the “BrainCap” (Falk-Minow Services, Herrsching-Breitbrunn, Germany), which is part of the MR-compatible EEG recording system “BrainAmp-MR” (Brainproducts Co., Munich, Germany). Electrode impedance was kept below 7 kΩ. Data were transmitted from the high-input impedance amplifier (250 Hz low-pass filter, 10 s time constant, 16-bit resolution, dynamic range 16.38 mV), which was placed directly behind the head coil inside the scanner room and connected to a computer located outside the scanner room via a fiber optic cable. Two additional electrodes were placed on the infraorbital ridge of the right eye for recordings of the vertical electrooculogram and on the left perivertebral part of the low back for acquisition of the electrocardiogram to control for heartbeat artifacts. The scanner (10 MHz sampling rate) was synchronized with the EEG amplifier (5 kHz sampling rate). Online correction of gradient artifacts based on the averaged artifact subtraction (AAS) algorithm was performed using RecView software (Brainproducts Co.), enabling visual inspection of EEG quality throughout the recording.

Gradient artifacts as a result of electromagnetic distortion of the EEG through static and dynamic magnetic field during MR data acquisition and ballistocardiogram artifacts were removed offline using the AAS method as implemented in the BrainVision Analyzer 1.05 software (Brainproducts Co.) (Allen et al. 1998, 2000). After artifact correction, the data were downsampled to 250 Hz and then filtered at 0.03–75 Hz.

VEP were analyzed at the Oz position of the international 10/20 system. Data were transformed to averaged reference. The EEG signal was corrected for DC drift, eye movements, and blinking (algorithm described by Gratton et al. 1983, as implemented in BrainVision Analyser). Artifacts were rejected automatically if the signal amplitude exceeded 100 μV; gradient increase exceeded 50 μV. The automatic rejection was checked by visual inspection. Only artifact-free trials entered further analysis. Averages were digitally low-pass filtered at 30 Hz (24 dB/octave roll-off) and segmented into epochs of 400 ms (100 ms pre- to 300 ms poststimulus). The 100 ms before the stimulus were taken as baseline. Averages were analyzed for peak-to-peak amplitudes of the maximal negative or positive deflections (N80–P100). The following time ranges were defined: N80, 60–110 ms, and P100, 80–120 ms (Odom et al. 2004). Peaks were detected automatically and confirmed manually by an experienced investigator who was blinded with regard to the diagnosis and condition. The typical positive and negative deflections could be clearly identified in each individual recording. The groups did not differ significantly with regards to the number of rejected trials (number of rejected trials in each block and subject: 20.2 ± 10.2 among migraine patients; 22.1 ± 9.8 among healthy subjects; analysis of variance [ANOVA]—nonsignificant). The entire recording session was divided into 2 blocks with an equal number of trials. Habituation of VEP was characterized as decrease in N80–P100 amplitude across blocks.
Magnetic Resonance Spectroscopy

$^1$H-MRS was carried out using a whole-body 3-T MR scanner and a standard head coil (Philips Achieva, Philips, Best, The Netherlands). Before $^1$H-MRS, we acquired a 3-D $T_1$-weighted structural MRI scan of the whole brain using a magnetization prepared rapid gradient echo sequence (1 mm slice thickness, 208 × 208 matrix, 150 slices, field of view = 208 mm, time echo [TE] = 3.6 ms, time repetition [TR] = 7.8 ms, flip angle = 8°, NSA = 2). The structural MRI scan was used to position the voxel of interest (VOI) in the visual cortex, centered on the calcarine sulcus, including the primary and secondary visual cortices (Fig. 1; Brodmann’s areas 17, 18, and 19). The VOI was chosen based on a functional $^1$H-MRS study in migraine patients (Sarchielli et al. 2005) and our previous fMRI study (see PS and Moeller et al. 2009).

For spectroscopic acquisition, a single-voxel PRESS (point-resolved spectroscopy) pulse sequence was used with TE = 37 ms, TR = 2000 ms, repetitions (NSA) = 128, VOI 20 × 20 × 20 mm. Each acquisition block lasted 5 min. Water suppression was achieved by excitation prepulse. Spectra were analyzed automatically by using curve-fitting software (Philips) with zero filling, Fourier transformation, baseline, and eddy current correction and normalized to water-yielding quantification of metabolites of interest. There were no spectra showing abnormal baseline. For all subjects and conditions, the peak areas (in arbitrary units) of the NAA (1.9–2.1 ppm), creatine (Cr, 3.03–3.94 ppm), and the Glx (unresolved glutamine/glutamate/γ-aminobutyric acid [GABA], 2.2–2.4 ppm) were measured and used for statistical analysis. Other peaks were not considered as they have not been shown to play a significant role in the pathogenesis of migraine in previous studies. No lactate peak (1.2–1.5 ppm) was observed in healthy subjects or migraine patients, presumably due to the low sensitivity of MRS toward lactate measurements at 3 T (Lange et al. 2006). Results are given as ratios of NAA and Glx to Cr, since the Cr peak is stable and can thus be used as internal reference (Deicken et al. 2000). It has been shown that the Glx peak is dominated by glutamate, while the effects of glutamine and GABA included in the Glx peak play a minor role (Auer et al. 2000).

Transcranial Direct Current Stimulation

During tDCS, subjects were seated in a reclining chair in a dark room and were told to keep their eyes closed. Tonic tDCS was continuously applied for 10 min at a current strength of 1 mA with a ramp-up and ramp-down time of 10 s. The direct currents were transferred via a saline-soaked pair of surface sponge electrodes (35 cm²), which were connected to a battery-driven constant current stimulator (Schneider Electronics, Gleichen, Germany). For cathodal tDCS, the cathode was placed at Oz and the reference over Cz. For anodal tDCS, the current flow was reversed. This electrode position and stimulation duration had been shown to be optimal for stimulation of the visual cortex in a number of previous studies (Antal et al. 2008). Constant current flow was measured by a voltmeter and controlled by the experimenter. All subjects experienced slight itching under the electrodes during both cathodal and anodal tDCS.

Statistical Evaluation

SPSS for Windows Version 17.0 (SPSS Inc., Chicago, IL) was used for the statistical analyses. All data were normally distributed
(Kolmogorov–Smirnoff tests, $P > 0.8$) and characterized by homogeneous variances ($F$-test). Normalized MRS peaks of NAA and Glx (NAA/Cr ratio, Cr, and Glx/Cr ratio) were entered into a 3-way repeated-measures ANOVA with a between-subject main factor "group" (migraine patients vs. healthy subjects) and 3 within-subject main effects: "polarity of tDCS" (anodal tDCS vs. cathodal tDCS), "time" of measurement relative to tDCS (before vs. after tDCS), and "PS" (no PS vs. PS). Changes in VEP amplitude (N80–P100) were analyzed using a similar ANOVA without the effect PS. In the assessment of the habituation effect, the ANOVA model was extended to include the additional factor "habituation" (first block vs. second block of measurement). The Greenhouse–Geisser method was used to correct for nonsphericity. If the $F$ value was significant, pairwise 2-tailed $t$-tests were used for post hoc analysis to estimate differences between conditions. A $P$ value of <0.05 was considered significant. To test for a linear relation between relative changes in VEP amplitude and Glx/Cr ratio as a result of tDCS, we calculated the Pearson product-moment correlations.

**Results**

None of the subjects reported any adverse events during or after the experiments. In particular, no aura was induced by visual stimuli in any of the experimental sessions. Table 1 shows NAA/Cr, Glx/Cr, and Cr values across all conditions (mean ± SD). Figure 2 demonstrates differences of Glx/Cr ratio between groups and conditions. The analysis of NAA/Cr ratios and Glx/Cr ratios, which were obtained during the first and second experimental session (independently on the first anodal or cathodal stimulation), revealed significantly higher Glx/Cr ratios in migraine patients compared with healthy subjects (Fig. 2B, $t_{18} = –2.26; P = 0.036$ for the first session and $t_{18} = –2.13; P = 0.047$ for the second session). Between-group comparison of Glx/Cr ratios for baseline recordings before the anodal or cathodal stimulation showed significantly higher ratios in the

| Table 1 |
|-----------------|-----------------|-----------------|
| NAA/Cr, Glx/Cr ratios, and Cr peaks in different conditions and both groups of participants |

**Figure 2.** Results of MRS in both groups of healthy subjects and migraine patients. (A) Examples of typical spectra with metabolites, which were analyzed in this study. (B) Glx/Cr ratios in healthy subjects and patients with migraine obtained during the first and second measurement before any stimulation (first and second baseline conditions). Note the significantly increased Glx/Cr ratios in migrainers, independent of the recording sequence. (C and D) Changes of Glx/Cr ratios represented in percentage of change compared with baseline, under PS and after anodal (C) and cathodal (D) tDCS.
migraine group before the anodal tDCS ($t_{18} = -2.68; P = 0.015$) but only tended to have higher ratios in migraineurs before cathodal tDCS ($t_{18} = -1.48; P = 0.155$; see Table 1). The baseline values for the NAA/Cr ratio and of Cr did not differ between the groups, neither in the comparison of both sessions nor for both baseline recordings.

Figure 2C,D illustrates the relative changes in Glx/Cr ratio caused by PS and tDCS in healthy subjects and patients with migraine. Patients and healthy subjects showed marked differences in their response to visual and DC stimulation. In healthy subjects, Glx/Cr ratios were not altered by PS. Conversely, patients demonstrated a decrease in Glx/Cr ratio in response to the first PS procedure preceding tDCS. Anodal tDCS caused a relative increase in Glx/Cr ratio and cathodal tDCS a relative Glx/Cr decrease in healthy controls. This DC effect on Glx/Cr ratio was attenuated in patients with migraine, especially in the anodal tDCS condition. Accordingly, ANOVA revealed a main effect of “polarity of tDCS” ($F_{1,18} = 4.859; P = 0.041$) and interactions between PS and “group” ($F_{1,18} = 4.859; P = 0.041$); “polarity of tDCS” and PS ($F_{1,18} = 10.967; P = 0.004$); “polarity of tDCS,” PS, and group ($F_{1,18} = 4.858; P = 0.04$); and “polarity of tDCS,” PS, “time of measurement,” and group ($F_{1,18} = 9.24; P = 0.007$). There were also statistical trends toward a main effect for polarity ($F_{1,18} = 3.602; P = 0.074$) and between polarity of tDCS and PS ($F_{1,18} = 4.073; P = 0.059$).

In healthy subjects, pairwise post hoc comparisons demonstrated that in the session with anodal tDCS (Fig. 2C), the first PS did not change the Glx/Cr ratio. Furthermore, anodal tDCS produced a significant increase in the Glx/Cr ratio compared with baseline ($t_9 = -2.49; P = 0.034$), which then returned to baseline after the second PS ($t_9 = 2.67; P = 0.026$). In the session in which cathodal tDCS was given (Fig. 2D), PS did not produce significant changes in the Glx/Cr ratio. Subsequently, the Glx/Cr ratio decreased after the cathodal tDCS ($t_9 = 2.302; P = 0.047$) and showed a tendency to return to baseline under the second PS ($t_9 = -2.19; P = 0.058$).

In migraine patients, pairwise post hoc comparisons revealed that in the session with anodal tDCS (Fig. 2C), the first PS reduced the Glx/Cr ratio in the occipital cortex ($t_9 = 5.503; P < 0.001$). This relative reduction persisted throughout the rest of the session with no further modulation of the Glx/Cr ratio by anodal tDCS (baseline vs. anodal tDCS: $t_9 = 4.429; P = 0.002$) or the second PS (baseline vs. second PS: $t_9 = 4.207; P = 0.002$). In the session with cathodal tDCS (Fig. 2D), the first PS caused a slight but nonsignificant reduction of the Glx/Cr ratio ($t_9 = 0.92; P = 0.382$), which reached significance after additional cathodal tDCS (baseline vs. tDCS: $t_9 = 2.365; P = 0.042$) and did not change after the second PS.

Neither patients nor controls showed significant changes in the NAA/Cr ratio or Cr in any of the experimental conditions.

Figure 3. Amplitude of VEP in healthy subjects and migraine patients. (A and B) Grand averages of N80-P100 amplitudes shown for both groups (A—healthy controls, B—migraineurs) and both stimulation polarities: “black” before and “red” after anodal tDCS, as well as “green” before and “blue” after cathodal tDCS. (C) Bar graph representing changes in the N80-P100 amplitude (means and SDs) for both groups and both stimulation polarities. (D) Significant correlation between changes in the N80-P100 amplitude and changes in the Glx/Cr ratios after both anodal and cathodal tDCS.
Figure 3 summarizes the VEP data. At baseline, migraine patients had significantly higher N80–P100 amplitudes compared with healthy subjects, independent of the order of session (first session $t_{18} = -2.86; P = 0.01$ and second session $t_{18} = -3.966; P = 0.001$) or the type of session (baseline before anodal tDCS $t_{18} = -2.56; P = 0.02$ and baseline before cathodal tDCS $t_{18} = -4.581; P < 0.001$). In addition, tDCS had a different modulatory effect on the VEP amplitude in patients and controls. In healthy subjects, anodal tDCS increased the N80–P100 amplitude, whereas cathodal tDCS suppressed the N80–P100 amplitude (Fig. 3C). In patients with migraine, the N80–P100 amplitude did not change regardless of the polarity of tDCS (Fig. 3C). The ANOVA confirmed the differences in VEP amplitude. Using the N80–P100 amplitude as a dependent variable, the ANOVA revealed significant interactions "polarity × group" ($F_{1,18} = 11.411; P = 0.003$) and "polarity × tDCS" ($F_{1,18} = 8.847; P = 0.012$). Post hoc analysis demonstrated that, in the group of healthy subjects, the anodal tDCS led to an increase in the N80–P100 amplitude ($t_9 = -2.276; P = 0.023$), while cathodal tDCS caused a decrease in N80–P100 amplitude ($t_9 = 3.469; P = 0.007$). No consistent tDCS-induced changes in N80–P100 amplitude were found in the group of migraine patients.

We tested whether the interindividual variation of the VEP amplitude changes after tDCS correlated with the interindividual variation of tDCS-induced changes in the Glx/Cr ratio as revealed by $^1$H-MRS. Only healthy subjects showed a correlation between tDCS-induced changes in N80–P100 amplitude and the Glx/Cr ratio in the occipital cortex (Fig. 3D). No such correlation was present in patients with migraine. There were also no significant correlations between individual variations in baseline Glx/Cr ratios and VEP amplitudes both in patients with migraine and healthy controls.

Figure 4 illustrates the relative habituation of the N80–P100 amplitude for both groups and experimental sessions. As described above, patients showed a relative increase in VEP amplitude compared with control subjects, which was consistent across experimental conditions ($P < 0.01$ for all comparisons). Healthy subjects displayed pronounced habituation with a reduction in the N80–P100 amplitude from the first to the second block at baseline (Fig. 4A). The habituation of VEP amplitude was missing after cathodal tDCS, whereas habituation became more pronounced after anodal tDCS (Fig. 4A). Patients with migraine lacked consistent habituation when the second half of the recordings was compared with the first half for a given VEP measurement (Fig. 4B). These differences in VEP habituation were confirmed by the ANOVA, which showed a main effect of group ($F_{1,18} = 14.608; P = 0.001$) and “habituation” ($F_{1,18} = 4.511; P = 0.048$), as well as interactions between group and habituation ($F_{1,18} = 18.755; P < 0.001$); “polarity” and habituation ($F_{1,18} = 15.173; P = 0.001$); and polarity, “tDCS,” and habituation ($F_{1,18} = 21.289; P < 0.001$).

Post hoc t-tests revealed significant VEP habituation in healthy subjects for both baseline conditions (block 1 vs. block 2: baseline before anodal $t_9 = 5.582; P < 0.001$ and cathodal $t_9 = 3.462; P = 0.007$ stimulation) and after anodal tDCS ($t_9 = 6.209; P < 0.001$). In contrast to anodal tDCS, which tended to increase VEP habituation, VEP habituation was no longer present after cathodal tDCS ($t_9 = -0.564; P = 0.586$). There were no significant changes in the N80–P100 amplitude between the blocks of recording in all conditions in the group of migraine patients, indicating that VEP habituation was consistently absent. In healthy subjects, there was a weak correlation between individual changes in the N80–P100 amplitude in the first block of recording with individual changes in Glx/Cr ratio after tDCS ($r = 0.32; P = 0.047$). No other correlations between VEP and MRS measurements were found.

**Discussion**

**Cortical Neurotransmitter Concentrations and Evoked Potentials**

This study provides evidence for altered glutamatergic function in migraine with aura. At rest, migraineurs showed increased Glx/Cr ratios relative to healthy controls as revealed by $^1$H-MRS. This difference was not related to fluctuations of the Cr peak, which remained stable throughout all conditions in both groups (Table 1). The demonstration of an increased Glx/Cr ratio corroborates the notion of excessive glutamate-mediated excitation in migraine. A link between altered glutamate signaling and migraine is indirectly suggested by experiments on CSD, which is believed to represent an initial manifestation of a migraine attack (Somjen 2001; Peeters et al. 2007; Tottene et al. 2009). CSD can be induced by N-methyl-D-aspartate (NMDA), by magnesium depletion, which releases the NMDA receptor channel, and can be blocked by NMDA antagonists (Mody et al. 1987; Lauritzen and Hansen 1992; Nellgard and Wieloch 1992; Peeters et al. 2007). Accordingly, several groups have found
high levels of glutamate in plasma, cerebrospinal fluid, and erythrocytes in the headache-free interval and during the at-
tack of patients with migraine with and without aura (Ferrari et al. 1990; D’Andrea et al. 1991; Martinez et al. 1993; D’Eufemia et al. 1997; Alam et al. 1998). Furthermore, transcranial mag-
netic stimulation studies have revealed enhanced intracortical facil-
itation in migraine, which is closely related to glutamate-
genic activity (Siniatchkin et al. 2007; Conte et al. 2010). More-
over, NMDA receptor antagonists and modulators seem to
prevent aura and are able to treat attacks in migraine patients
(Kaube et al. 2000; Marin and Goadsby 2010). Finally, anti-
epileptic drugs acting on the glutamatergic system have a
prophylactic effect in migraine (Vikelis and Rapoport 2010).
Together, these studies and our results suggest that glutama-
teric systems play a significant role in the pathogenesis of
migraine with aura.

At baseline, migraine patients presented with increased VEP
amplitudes and Glx/Cr ratios relative to healthy subjects. This
shows that migraine with aura is associated with both altered
 glutamatergic neurotransmission (as indexed by the Glx/Cr
ratio in the $^1$H-MRS spectrum) and abnormal cortical in-
formation processing (as indexed by the VEP amplitude). Since
there were no significant correlations between absolute Glx/Cr
ratios and VEP amplitudes, it is unlikely that the increase in
Glx/Cr ratio determines the increase in VEP amplitude in
migraine with aura. However, we cannot draw firm conclusions
given the relatively small number of patients in our study.

Only healthy subjects showed a correlation between the
tDCS-induced changes in Glx/Cr ratio and VEP amplitude,
while patients did not show such correlation. A combination of
factors might account for the decoupling of changes in cortical
information processing and glutamine signaling in migraine.
Excessive changes of cortical excitability and glutamatergic
function under tDCS might have produced ceiling or floor
effects on VEP (Schoenen 1996). Another factor might be the
fast depletion of glutamate following its increased consumption
in migraine (see Altered responsiveness of the visual cortex in
migraine). It is also conceivable that other neurotransmitters
contribute more strongly to the regulation of VEP amplitude,
for example, dopamine and serotonin, which play an important
role in the pathogenesis of migraine (Mascia et al. 1998; Hamel
2007). Abnormal cortical GABA-ergic inhibition, which has
been observed in migraine (Bigal et al. 2008; Siniatchkin et al.
2009), might also play a role. Unfortunately, cortical GABA
concentrations could not be investigated in this study because
of a nonappropriate MRI sequence. These issues remain to be
addressed in future studies.

Modifiability of the Visual Cortex in Healthy Subjects

In healthy subjects, both Glx/Cr ratio and amplitudes of VEP
changed according to the polarity of the tDCS. The $^1$H-MRS
changes under cathodal tDCS are in good agreement with a
recent study by Stagg et al. (2009), which demonstrated a
significant decrease in Glx/NAA ratio during cathodal tDCS. Yet,
Glx/Cr increased following anodal tDCS in our study, whereas
the Glx/NAA ratio did not change significantly in the study of
Stagg et al. (2009). The discrepancies between the studies may
be explained by methodological differences. First, we applied
tDCS shortly after PS, which is known to influence cortical ex-
citability (Vincent et al. 2005). Although the effect of the first PS
in healthy subjects was rather modest, a possible gating effect of
PS on subsequent tDCS cannot be ruled out. Second, the study of
Stagg et al. (2009) investigated glutamatergic function during
tDCS, whereas we measured the Glx/Cr ratio ca. 5 min after the
end of tDCS. The relative timing of $^1$H-MRS with respect to tDCS
may indeed be relevant. Stagg et al. (2009) demonstrated that
tDCS of both modalities may exert a long-lasting aftereffect and
that changes in the Glx/NAA ratio are more pronounced after
stimulation than during tDCS. Despite these methodological
differences, tDCS had a pronounced effect on the occipital Glx
concentration in both studies.

Both anodal and cathodal tDCS have the potential to alter
excitability of the visual cortex in humans (Antal et al. 2006;
Chadaide et al. 2007). In agreement with previous studies, we
found an increase in the N80–P100 amplitude of VEP after
anodal and a decrease in amplitude after cathodal tDCS of the
occipital cortex. The individual change in the Glx/Cr ratio
measured shortly after tDCS in the occipital cortex predicted
the individual change in VEP amplitude. The stronger the
increase in Glx/Cr ratio with anodal tDCS the larger the
increase in N80–P100 amplitude was and vice versa. These
correlations suggest that the tDCS-induced changes in gluta-
mergic function contributed to the alteration in responsiveness
to visual stimulation.

Occipital tDCS also had a lasting, polarity-specific effect on
the habituation of VEP amplitude. While anodal stimulation
increased habituation, VEP habituation was decreased after
cathodal tDCS. The magnitude of VEP habituation is influenced
by the VEP amplitude in the first block of recording: the higher
the amplitude in the first block the more pronounced the
habituation was (Bohotin et al. 2002; Schoenen 2006; Siniatch-
kin et al. 2007). The relative changes in habituation after tDCS
may be explained by tDCS-induced changes in VEP amplitudes
in the first block of recording. Indeed, anodal tDCS led to an
increase and cathodal tDCS to a reduction of the N80–P100
amplitude in the first block. We argue that the polarity-specific
effects of tDCS on VEP habituation represent an adaptive
regulatory mechanism meant to balance excitatory and in-
hibitory activity in the visual cortex (Brighina et al. 2009;
Coppola et al. 2009).

There is another adaptive mechanism, which may explain
the results in healthy subjects in our study. While PS only had
a modest effect on Glx/Cr ratio before tDCS, PS induced
changes in the Crx/Cr ratio after tDCS, which depended on the
polarity of the preceding tDCS session. When glutamatergic
neurotransmission was increased after anodal tDCS or de-
creased after cathodal, the PS changed the Glx/Cr ratio in the
opposite direction. Therefore, the PS exerted a homeostatic
effect on the Glx/Cr ratio, which depended on the preceding
effect of tDCS on glutamatergic neurotransmission. This de-
pendence of PS-induced neuromodulation on cortical excitability
as a function of preceding excitability changes can be
explained in the framework of “homeostatic metaplasticity”
(Bienenstock et al. 1982; Bear et al. 1987; Bear 2003; Lang et al.
2004, 2007; Siebner et al. 2004). For example, in the visual
cortex, anodal tDCS led to a transient decrease in phosphene
threshold, and subsequent 5-Hz repetitive transcranial mag-
netic stimulation (rTMS) induced an earlier return of the
phosphene threshold back to baseline, and cathodal tDCS
produced a short-lasting increase in phosphene threshold,
although the regulatory effect of subsequent rTMS after
cathodal tDCS was rather moderate (Lang et al. 2007).
Homeostatic regulation of cortical excitability has been first
conceptualized by the influential Bienenstock–Cooper–Munro
Altered Responsiveness of the Visual Cortex in Migraine

Migraine patients were characterized by an altered response profile of the visual cortex. The first PS caused a reduction in the Glx/Cr ratio. This reduced glutamatergic neurotransmission remained stable and unchanged after both anodal and cathodal tDCS. Moreover, the second PS had no effect on the reduced Glx/Cr ratio. There were also minimal and non-significant changes in the VEP amplitude and habituation after tDCS. We were unable to demonstrate any adaptive effect that was seen in healthy subjects. Patients lacked habituation as a compensation for the increased preactivation level as well as homeostatic plasticity, indicating deficient regulatory mechanisms of cortical excitability in migraine. These results are in line with previous findings. Bohotin et al. (2002) have shown that repetitive TMS may alter levels of cortical preactivation and excitability in healthy subjects and migraine patients in a different way. Brighina et al. (2002) have demonstrated a paradoxical effect of repetitive TMS on intracortical inhibition/facilitation in migraine. While intracortical facilitation decreased significantly in controls after inhibitory 1 Hz rTMS, it increased in migraineurs. In migraine with and without aura, Chadaide et al. (2007) demonstrated an altered response of visual cortex to cathodal tDCS, which was attributed to deficient inhibitory cortical processes, especially in migraine with aura. Antal et al. (2008) provided the first evidence that homeostatic plasticity is altered in migraineurs. In both healthy subjects and migraine patients, a short train of low-intensity 5-Hz rTMS antagonized the suppression of the amplitude of motor-evoked potentials following cathodal tDCS. In contrast, the homeostatic effects of 5-Hz rTMS differed between groups when rTMS was given after anodal tDCS.

It seems likely that the altered modifiability of cortical excitability in migraine and insufficient homeostatic plasticity are associated with altered glutamatergic function. In migraine patients, tDCS decreased the Glx/Cr ratio, regardless of the polarity of tDCS. A similar effect was observed after 10-Hz TMS in healthy subjects (Michael et al. 2003). This stimulation-induced reduction in the Glx/Cr was explained in terms of the Glx pool being as a result of induced neuronal currents and subsequent depolarization, an effect which has been demonstrated in animals (Zangen and Hyodo 2002). We propose that migraine is associated with an increased consumption of glutamate, which is quickly utilized by the first sequence of stimulation and cannot be effectively used for homeostatic regulation of cortical excitability. Abnormal homeostatic regulation may be key to abnormal information processing (lack of habituation) and cortical excitability in migraine but may also play a role in causing abnormal visual perception (Chronicle and Mulleners 1996; Siniatchkin et al. 2009), stress vulnerability (Siniatchkin, Averkina, Andrasik, et al. 2006; Siniatchkin, Averkina, and Gerber 2006; Sauro and Becker 2009), and a number of comorbidities (Bruijn et al. 2010; Antonaci et al. 2011).

Limitations and Outlook

This study has some limitations, which have to be considered in future studies. The first limitation is related to the small number of patients and control subjects with an increased risk for false-negative findings (statistical type II error). This limitation may explain some moderate and nonsignificant changes, for example, the moderate increase of Glx/Cr ratio under the second PS and no changes of the ratio under the first PS in healthy controls. The second limitation regards the study design. The PS before tDCS might have primed the effects of tDCS, for example, by inducing homeostatic plasticity. However, the main focus of the study was directed to changes of VEP under tDCS and their relationship to changes in glutamatergic activity. This required the recordings of VEPs before and after tDCS. The study of homeostatic plasticity was only a secondary subordinate topic. If PS itself triggered homeostatic plasticity in visual cortex, the aftereffects induced by tDCS might reflect at least in part a homeostatic response to the first period of PS. Ideally, there should have been a control experiment without PS at least before tDCS to rule out an effect of the first PS on subsequent metaplasticity in the migraine group. Future studies need to study the homeostatic interactions between consecutive interventions in more detail to get a better understanding of how interventions that influence physiologically evoked cortical activity (e.g., PS) interact with protocols that transcranially stimulate the visual cortex (e.g., tDCS).

Another limitation relates to the interpretation of the changes in evoked potentials under tDCS. Here ceiling and floor effects might have contributed to the differences in the response pattern between patients and controls. Furthermore, the study focused only on changes of glutamate. The use of advanced 1H-MRS sequences that allow to reliably assess the GABA peak in the spectrum would have enabled us to study metabolic markers of excitatory glutamatergic and inhibitory GABAergic activity (Stagg et al. 2009), and this would have enabled deeper insight in the pathogenesis of migraine. Since we only studied patients suffering from migraine with aura, future studies need to be extended to patients with migraine without aura and especially with chronic migraine as similar changes of cortical excitability and information processing have been shown for these types of migraine too (Schoenen et al. 2003; Schoenen 2006).

Notes

The authors have no professional or financial affiliations that might be perceived as having biased the presentation. All the authors have agreed to be listed. The material has not been published and is not under consideration for publication elsewhere. Conflict of Interest: None declared.

References


