Hyperexcitability of the primary somatosensory cortex in migraine—a magnetoencephalographic study

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Summary
The excitability of the cerebral cortex in the interictal state of migraine appears to be fundamental in the brain’s susceptibility to migraine attacks. Subpopulations of cortical neurons are reported to have different physiological response properties to different interstimulus intervals (ISIs) and, hence, may be differentially altered or modulated in migraine. The aim of this study therefore was to evaluate response characteristics of temporally and spatially defined neuronal subpopulations in the cortex of migraineurs. To this end, we measured, by means of magnetoencephalography (37-channel neuromagnetometer), the response properties of the early components of the somatosensory evoked magnetic fields following electrical stimulation of the median nerve, the N20m and P35m, at ISIs ranging between 0.3 and 6 s. As a measure of the number of excited neurons underlying the N20m and P35m, we evaluated the root mean square (r.m.s.) of the deflections across all 37 channels at the corresponding latencies and the corresponding dipole moment of the equivalent current dipole (ECD strength). Twenty consecutive women with at least three migraine attacks/month (range 3–8/month) fulfilling the International Headache Society criteria and 20 age-matched healthy women were included in the study. In migraineurs, the r.m.s. and ECD strength of N20m was increased at all ISIs (r.m.s., $P < 0.05$; ECD strength, $P < 0.01$) and positively related to the mean attack frequency (r.m.s., $R_s = 0.6, P < 0.01$; ECD strength, $R_s = 0.5, P < 0.05$). In contrast, the r.m.s. and ECD strength of P35m did not differ significantly between migraineurs and control subjects and did not correlate significantly with the frequency of migraine attacks. Responses to different ISIs did not differ significantly between migraineurs and control subjects. The r.m.s. of N20m was stable for ISIs between 0.5 and 6 s and decreased significantly at an ISI of 0.3 s. In contrast, the r.m.s. of P35m decreased continuously as the ISI was decreased below 6 s and this reached significance for an ISI of $< 1$ s. Habituation of N20m or P35m, i.e. a decrease in response magnitude following repetitive stimulation over time, was not found in either the control subjects or in the migraineurs. It is concluded that the population of neurons in the primary somatosensory cortex underlying the N20m are hyperexcitable and that this hyperexcitability is linked to the frequency of migraine attacks. This hyperexcitability appears not to be related to habituation since habituation was not found in the control subjects. In contrast, the magnitude of P35m is not pathophysiologically linked to the interictal state of migraine. Furthermore, the cellular mechanisms causing ISI-dependent depression of N20m and P35m are not altered in migraine.

Keywords: migraine; magnetoencephalography; somatosensory evoked magnetic fields; primary somatosensory cortex

Abbreviations: ECD = equivalent current dipole; fT = femtotesla; ISI = interstimulus interval; MEG = magnetoencephalography; nAm = nanoamperemetre; r.m.s. = root mean square; SEF = somatosensory evoked magnetic field; SI = primary somatosensory cortex

Introduction

In migraine, abnormal pain processing of the trigeminovascular system during attacks is often associated with an abnormal processing of sensory information in the brain between the attacks. According to the contemporary concepts of migraine pathogenesis, the excitability of neurons in the visual cortex appears fundamental to the brain’s susceptibility to migraine attacks (Welch, 2003). Various interictal abnormalities of evoked and event-related potentials have been reported for different cortical areas (Ambrosini et al., 2003a). Cortical hyperexcitability in migraineurs is supported by increased amplitudes of visual evoked potentials (Diener et al., 1989; Shibata et al., 1997, 1998), deficient habituation of cortical evoked responses, i.e. a decrement of response amplitudes following repetitive stimuli over time (Kropp and Gerber, 1995; Schoenen, 1996; Kors et al., 1999), and an increased contingent negative variation (Schoenen et al., 1985; Kropp and Gerber, 1993). In contrast, studies showing an increased dependency of the amplitudes of auditory evoked potentials on the stimulus intensity (Wang et al., 1996; Ambrosini et al., 2003b) and a low first-block amplitude of visual and auditory evoked potentials (Afra et al., 2000) favour the hypothesis that deficient cortical habituation in migraineurs causes an interictal cortical hypoexcitability.

Such inconsistencies arising from the results of previous research on cortical excitability in migraine might be due in part to methodological differences with regard to stimulation techniques. Another source of variation may result from the variability of the signal parameter used for evaluation. In most evoked potential studies examining migraine, cortical excitability was evaluated from the peak-to-peak amplitude of the early components of the evoked potentials. However, the peak-to-peak amplitude represents a mixture of at least two sequentially activated neuronal generators which may be differentially affected in migraine.

A disadvantage of single-channel EEG studies is that the neuronal generators of each deflection in the recorded potential cannot be determined precisely. Dipole localization techniques favour multi-channel magnetoencephalography (MEG) due to the higher accuracy of MEG source localization compared with EEG when using the standard spherical head shape model (Barkley and Baumgartner, 2003). Hence, the response properties of spatially distinct neuronal populations within the cortex can be investigated better by MEG than by EEG (Hämäläinen et al., 1993). Because of this advantage, we used MEG to determine whether well characterized cortical generators, the short-latency components N20m and P35m of the somatosensory evoked magnetic fields (SEFs) following electrical stimulation of the median nerve, showed abnormal response properties in the interictal interval of migraine.

The N20m and P35m of the somatosensory evoked magnetic fields following electrical stimulation of the median nerve are evoked by tangentially oriented current sources which are located close together in area 3b of the primary somatosensory cortex (SI) contralateral to the side of stimulation (Wikström et al., 1996; Hoshiyama and Kakigi, 2001). Different directions and slightly different locations of the equivalent current dipoles (ECDs) of N20m and P35m indicate two separate neuronal generators. Another important difference between the generators of N20m and P35m lies in their response properties to different interstimulus intervals (ISIs) of median nerve stimulation (Wikström et al., 1996): the ECD strength of N20m is stable for ISIs between 5 and 0.5 s, whereas the ECD strength of P35m decreases with decreasing ISI throughout this range. Because of strong similarities between the ISI dependence of N20m and P35m and that of intracellularly recorded potentials from the SI, it was proposed that N20m represents a primary population excitatory postsynaptic potential (EPSP) and P35m corresponds to an early inhibitory postsynaptic potential (IPSP) (Wikström et al., 1996).

Although the model has not yet been confirmed pharmacologically, the proposed excitatory and inhibitory activity underlying N20m and P35m, respectively, rendered the model worthy of testing in the context of migraine. We examined the magnetic field strength of the N20m and P35m at different ISIs in order to find out if there were ISI-dependent or ISI-independent differences in the cortical excitability between migraineurs and healthy subjects. Additionally, we examined the phenomenon of habituation for N20m and P35m, i.e. a decrement of field strength over time, in order to evaluate whether possible differences in N20m and P35m between migraineurs and healthy subjects could be related to an abnormal cortical habituation in migraineurs.

For this study, we selected patients with frequent migraine attacks since we expected that cortical abnormalities in migraineurs would increase with the severity of migraine. Intercortical hyperexcitability of the neuronal network in the SI in migraineurs should be paralleled by either an increase in N20m or a decrease in P35m, or both. Such reciprocal changes of N20m and P35m would support an interictal hypoexcitability in the SI.

Methods

Subjects

Forty right-handed women (range of age: 20–62 years) participated in the study: 20 migraineurs (mean age ± SD 40 ± 15 years) and 20 age-matched (P > 0.2) volunteers (controls; mean age ± SD 34 ± 12 years). Fifteen women had migraine without aura (code 1.1) and five women had migraine with aura (code 1.2) (Headache Classification Committee of the International Headache Society, 1988). Patients were recruited consecutively from the out-patient pain facility of the Department of Neurology of the University of Erlangen-Nuremberg. Patients were included if they had reported migraine episodes for at least 4 years and had suffered from at least three migraine attacks per month during the last 3 months. Patients who had received preventive medication for migraine in the last 3 months were excluded. For treatment of migraine attacks, triptans or non-steroidal anti-inflammatory drugs in possible combination with antiemetics were permitted. Patients underwent recordings only on a day that they were without headache. All subjects were free of other neurological or systemic disease known to cause abnormalities of the somatosensory system.
system. Only women (from the medical staff and medical students) with no history of recurrent migraine-like headaches or ongoing medication were included in the control group. The study followed the tenets of the Declaration of Helsinki and was approved by the local ethics committee. Informed consent was obtained from all participants prior to the measurements.

**Electrical stimulation**

The right median nerve was stimulated with a constant-current stimulator (Digitimer DS7, Digitimer Ltd, Hertfordshire, UK) via a pair of felt-tip electrodes placed on the skin over the median nerve at the wrist. The electrical stimulus was a constant square-wave pulse of 0.2 ms duration. The intensity of each stimulus was adjusted to produce a reproducible non-painful twitch of the thumb, usually 2 mA above motor threshold. This stimulus intensity was sufficient to produce an SEF of saturated amplitude. The mean stimulus intensity was $7.2 \pm 1.8$ mA in controls and $7.2 \pm 1.5$ mA in migraineurs. The stimulus timing was controlled by the software of Biomagnetic Technologies, Inc., San Diego, CA (see below). In each run, 200 stimulus-related epochs were collected. For each subject, we performed one run per ISI. In all subjects, the ISI of the first run was 1 s. Thereafter, ISIs of 0.3, 0.5, 1.5, 2, 3 and 6 s were varied randomly within each patient. To control for stability of the recordings, the stimulation sequence was finished with a stimulation run at an ISI of 1 s.

**Data acquisition**

Subjects were placed on the right side of their body in a comfortable half-sitting position with the head stabilized by a vacuum pillow. Cortical responses were recorded with a 37-channel neuromagnetometer (Magnes II®; Biomagnetic Technologies, Inc.) in a magnetically shielded room. The detection coils of the neuromagnetometer were arranged in a uniformly distributed array of concentric circles over a spherically concave surface (144 mm diameter). Basically, the sensor arrays were placed with the centre above C3 according to the international 10–20 system. In all subjects, the sensor was in contact with the head.

Before the first stimulation run, all patients were instructed to keep their eyes closed and not to clench their teeth (in order to minimize artefacts evoked by eyelid movement and muscle activity) and to maintain their attention to all electrical stimuli over the whole run. Cerebral evoked magnetic fields were recorded in the time period between 110 ms before and at least 160 ms after the stimulus trigger with a bandwidth of 0.1–200 Hz. The sampling rate of the analogue signals was set at 520 Hz. For off-line analysis of the signals, the data were visually scanned for artefacts and only epochs without obvious artefacts (at least 180 artefact-free epochs) were averaged. DC-offset was calculated from the 100 ms time period before the stimulus (i.e. 10–110 ms) and subtracted from the recording in the subsequent period after the stimulus. To avoid reduction of the amplitude of the sharp N20m, the data were not filtered.

**MEG source localization**

Magnetic source imaging was performed as described previously (Druschky et al., 2000). Briefly, a sphere locally fitted to the head shape underneath the sensor was used as a volume conductor model. Afterwards, a mathematic process based on the Marquardt algorithm (Marquardt, 1963) was applied if the evoked magnetic fields showed an approximately bipolar distribution. The time interval between 15 and 40 ms after stimulus onset was analysed applying the single ECD model for each sampling time. For each localization, a correlation coefficient was calculated for the measured and the ideal magnetic dipole fields. The pre-selection criteria for the final dipole were a map correlation and a goodness of fit $>0.98$. Latencies were calculated in relation to the time of stimulation. In order to visualize results with respect to brain anatomy in selected subjects, the dipole locations were superimposed on MRI. A 1.5 T Magnetom (Siemens, Germany) was used and three skin markers were placed at fiducial points on the subject’s head. The location of the same fiducial points was also recorded relative to the neuromagnetometer position, thus establishing a common spatial reference for the transposition of three-dimensional coordinates between MEG and MRI data. In the Cartesian coordinate system, the y-axis passed through the pre-auricular points with the positive y-direction pointing to the left; the x-axis passed through the nasion with the positive x-direction pointing to the nasion; thus the positive z-direction was upwards.

**Data analysis**

The activation strength of N20m and P35m was evaluated both by the root mean square (r.m.s.; femtotesla, fT) of the deflections across all 37 channels at the point in time corresponding to the maximum signal deflection, and by the dipole moment of the corresponding ECDs (ECD strength: nanoamperemes, nAm). Latencies of N20m and P35m were determined at the corresponding r.m.s. maxima in the time interval between 15 and 40 ms after stimulus onset. While N20m represents the first r.m.s. maximum between 15 and 21 ms, the P35m was evaluated at the r.m.s. maximum between 26 and 40 ms. Additionally, the direction of ECD for P35m was checked for inverse direction as compared with the ECD of N20m in the contour display. R.m.s. values were preferred for response evaluation because of their lower variability in recordings with reduced signal-to-noise ratio in comparison with ECD strength. To control for effects of the distance between the magnetometer and the subject’s head, we additionally evaluated the corresponding ECD strength.

Components of the SEF later than 40 ms were not evaluated since local maxima of their deflections could not be clearly determined in every measurement.

The habituation of N20m and P35m was evaluated from the 200 cortical evoked responses of the first measurement with an ISI of 1 s. Ten consecutive blocks of 18–20 artefact-free cortical responses were averaged separately and the r.m.s. values were normalized to the first block ([r.m.s. of block 1–10) $\times 100$/r.m.s. of block 1].

**Statistics**

T-tests for dependent or independent samples were used to test for differences in continuous variables. Differences in r.m.s. and ECD strength of N20m and P35m between migraineurs and controls were evaluated by multiple analysis of variance (ANOVA) with ISI as the within-subject factor (repeated measures) and group and age (categorized by quartiles) as between-subject factors. Effects of ISI or habituation on the r.m.s. of N20m and P35m were evaluated by multiple ANOVA (ISI or block number and age as between-subjects factors) and post hoc comparisons (LSD test). Correlation between electrophysiological (mean value of measurements at ISIs of 0.5, 1, 1.5, 2, 3 and 6 s) and clinical data were evaluated by non-parametric statistics (Spearman rank correlation coefficient $R_s$); correlations between parametric data were made using a Pearson’s correlation ($r$). All tests were two-tailed, and statistical significance was determined at an $\alpha$ level of 0.05. Statistical analyses were performed with the SPSS 11.5 software package. If not indicated otherwise, group data are presented as mean $\pm$ SD.
Results

Clinical data

Clinical data regarding the course and the treatment of migraine are summarized in Table 1. Duration of the migraine disease was correlated with the duration of migraine attacks \((R_s = 0.6, P < 0.01)\), but not to the frequency of migraine attacks in the last 3 months \((R_s = 0.2, P = 0.5)\). Two of the patients suffered from migraine headache on 15 and 16 days/month, respectively. These patients consumed 10 and 12 single doses of triptans/month, respectively, for longer than 1 year and did not report an increase of their attack frequency. In the other patients, the total days with migraine headache was <15/month.

Dipole locations and latencies of N20m and P35m

N20m and P35m were recorded in all subjects. Cortical responses were localized in the hand area of the SI contralateral to the side of stimulation. An original recording and source localization are shown in Fig. 1.

In all subjects, the magnetic field patterns were bipolar, with the ECDs of N20m and P35m pointing in different directions (Fig. 1B). The angle between the ECD of N20m and P35m (evaluated from recordings at an ISI of 1 s) did not differ \((P > 0.8)\) between migraineurs \((167 \pm 14^\circ)\) and control subjects \((169 \pm 8^\circ)\). The mean peak latency and ECD coordinates of N20m and P35m did not differ \((P > 0.2)\) between migraineurs and control subjects and are given in Table 2. Differences between the coordinates of N20m and P35m indicate that the generator of P35m was located at a site in the cortex 4.7 \(\pm\) 4.4 mm more cranial \((z\text{-direction}, P < 0.001)\) and 2.9 \(\pm\) 6.7 mm more medial \((y\text{-direction}, P < 0.05)\) to the N20m generator. The location difference in the sagittal \((x\text{-direction}, 0.3 \pm 3.6 mm)\) plane was not significant.

R.m.s. and ECD strength of N20m and P35m

The r.m.s. (Fig. 2A, Table 3) and ECD strength (Fig. 3A, Table 3) of N20m were significantly higher in migraineurs than in the control subjects. In contrast, the r.m.s. (Fig. 2B, Table 3) and ECD strength (Fig. 3B, Table 3) of P35m did not differ significantly between groups.

Within the groups of migraineurs and control subjects, age of the subjects did not correlate significantly with the r.m.s. values (migraineurs, \(r = 0.3, P > 0.1\); control subjects, \(r = 0.1\),

Table 1  Clinical data of the migraineurs, mean ± SD (range)

| Duration of the migraine disease (months) | 271 ± 158 (48–564) |
| Duration of the migraine attacks (h)     | 39 ± 19 (8–72)     |
| Average frequency of the migraine attacks/month in the last 3 months | 4.8 ± 1.7 (3–8) |
| Average number of days with migraine headache/month in the last 3 months | 8 ± 4 (3–16) |
| Average number of single doses of triptans/month \((n = 17)\) in the last 3 months | 7.3 ± 3.2 (3–12) |
| Average number of single doses of non-steroidal anti-inflammatory drugs*/month \((n = 4)\) in the last 3 months | 14.5 ± 8.5 (2–20) |

*Equivalents of 1 g of acetylsalicylic acid.
Table 2 Peak latencies (ms) and coordinates (mm) of the equivalent current dipoles of the early components of the somatosensory evoked magnetic fields at an ISI of 1 s in control subjects (n = 20), migraineurs (n = 20) and calculated for all subjects (n = 40), (mean ± SD).

<table>
<thead>
<tr>
<th>Deflection</th>
<th>Group</th>
<th>Latency (ms)</th>
<th>x (mm)</th>
<th>y (mm)</th>
<th>z (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N20m</td>
<td>Control subjects</td>
<td>17.7 ± 1.9</td>
<td>1.5 ± 9.2</td>
<td>43.8 ± 5.3</td>
<td>84.0 ± 7.0</td>
</tr>
<tr>
<td></td>
<td>Migraineurs</td>
<td>18.0 ± 1.3</td>
<td>−1.8 ± 7.0</td>
<td>41.2 ± 5.3</td>
<td>84.5 ± 5.7</td>
</tr>
<tr>
<td></td>
<td>All subjects</td>
<td>17.9 ± 1.6</td>
<td>−0.2 ± 8.2</td>
<td>42.4 ± 5.4</td>
<td>84.3 ± 6.3</td>
</tr>
<tr>
<td>P35m</td>
<td>Control subjects</td>
<td>31.0 ± 2.8</td>
<td>1.4 ± 10.0</td>
<td>38.7 ± 4.8</td>
<td>88.1 ± 7.4</td>
</tr>
<tr>
<td></td>
<td>Migraineurs</td>
<td>31.9 ± 3.8</td>
<td>−1.3 ± 6.7</td>
<td>39.9 ± 4.6</td>
<td>89.5 ± 6.9</td>
</tr>
<tr>
<td></td>
<td>All subjects</td>
<td>31.5 ± 3.3</td>
<td>−0.0 ± 8.5</td>
<td>39.3 ± 4.7</td>
<td>88.8 ± 7.1</td>
</tr>
</tbody>
</table>

Data did not differ between control subjects and migraineurs (P > 0.2, independent t test).

Table 3 Results of the repeated measures ANOVA for group and age effects on r.m.s. (fT) and ECD strength (nAm) of N20m and P35m

<table>
<thead>
<tr>
<th>Deflection</th>
<th>Between-subject factor</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N20m, r.m.s. (fT)</td>
<td>Group</td>
<td>6.5</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>0.5</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>Group × age</td>
<td>0.2</td>
<td>0.89</td>
</tr>
<tr>
<td>N20m, ECD strength (nAm)</td>
<td>Group</td>
<td>7.8</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>0.5</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>Group × age</td>
<td>0.2</td>
<td>0.89</td>
</tr>
<tr>
<td>P35m, r.m.s. (fT)</td>
<td>Group</td>
<td>2.6</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>1.3</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>Group × age</td>
<td>1.2</td>
<td>0.33</td>
</tr>
<tr>
<td>P35m, ECD strength (nAm)</td>
<td>Group</td>
<td>1.6</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>1.3</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>Group × age</td>
<td>1.2</td>
<td>0.31</td>
</tr>
</tbody>
</table>

P > 0.6) (Fig. 4) nor with the ECD strength of N20m (migraineurs, r = 0.1, P > 0.7; control subjects, r = −0.1, P > 0.6). However, over all subjects, there was a non-significant tendency for a correlation between age and r.m.s. of N20m (r = 0.3, P > 0.06), but not between age and ECD strength of N20m (r = 0.1, P > 0.4). Age did not correlate with r.m.s. value or ECD strength of P35m in any of these groups.

In the subgroup of two patients who were close to chronic migraine and possible triptan overuse, there were no consistent high N20m strengths. The first patient suffered migraine headache 15 days/month (attack frequency, 5/month; attack duration, 72 h) and had an N20m strength of 110 fT (14 nAm). The other patient suffered migraine headache 16 days/month (attack frequency, 8/month; attack duration, 48 h) and showed a N20m strength of 171 fT (36 nAm). Neither r.m.s. nor ECD strength of N20m and P35m differed significantly between migraineurs with and without aura (independent t test).

The effect of ISI on the r.m.s. and ECD strength of N20m and P35m did not differ significantly between migraineurs and control subjects (Figs 2 and 3). The statistical analysis for changes of r.m.s. values is shown in Table 4. When the ISI was shortened from 6 to 0.3 s, the r.m.s. and ECD strength of...
N20m was stable down to an ISI of 0.5 s. At an ISI of 0.3 s, the r.m.s. of N20m decreased \((P < 0.01)\), whereas calculation of the corresponding ECD strength values produced inappropriately high values in four subjects with relative low signal-to-noise ratios. Therefore, the group effects were not evaluated for the ECD strength of N20m at an ISI of 0.3 s. Contrary to N20m, the r.m.s. and ECD strength of P35m decreased continuously for ISIs <6 s, and this effect reached significance for ISIs of \(\leq 1\) s \((P < 0.05)\). At an ISI of 0.3 s, the P35m could no longer be recorded in four migraineurs and five control subjects.

Compared with the r.m.s. values of the first stimulation run (ISI of 1 s), the r.m.s. value of the last stimulation run (time interval 62 ± 20 min) showed small variation (N20m, migraineurs, 1 ± 20%; N20m, control subjects, −3 ± 20%; P35m, migraineurs, −10 ± 17%; P35m, control subjects, −9 ± 17%). These variations did not differ \((P > 0.7)\) between groups.

Relationship of N20m and P35m to clinical data

The r.m.s. and ECD strength of N20m were strongly correlated with the mean frequency of migraine attacks during the last 3 months \((r_s = 0.6, P < 0.01); \) ECD strength, \(r_s = 0.5, P < 0.05\) \((\text{Fig. 5A})\). When the patients were split into two groups according to the median of their attack frequency, the r.m.s. of N20m was higher \((P < 0.05)\) in patients with attack frequencies of \(>5\) attacks/month \((n = 7)\) in comparison with those with three and four attacks/month \((n = 13)\). In a subgroup of patients with three and four attacks/month, the r.m.s. of N20m \((113 ± 33 \mu T, n = 13)\) did not differ \((P = 0.09)\) from the values of the control subjects \((91 ± 36 \mu T)\).
relationship between the r.m.s. of N20m and the duration of migraine attacks ($R_s = 0.4$, $P = 0.07$) was not significant (Fig. 5B). The r.m.s. of N20m was not significantly correlated with the duration of the migraine disease ($R_s = 0.2$, $P = 0.3$) nor with the number of triptans or analgesics consumed per month ($R_s = 0.1$, $P = 0.7$).

The r.m.s. of P35m was not significantly related to the frequency or duration of the migraine attacks (Fig. 5C and D) nor to any of the other clinical parameters.

**Habituation of N20m and P35m**

The phenomenon of habituation of N20m and P35m was evaluated using a relative value. The percentage change of N20m or P35m did not vary significantly over blocks and did not differ significantly between migraineurs and control subjects (Table 5, Fig. 6). Thus, we did not find any significant habituation in any of the SEF components nor differences between the two groups.

**Table 5** Results of the ANOVA for block and group effects on r.m.s. of N20m and P35m in the evaluation of habituation

<table>
<thead>
<tr>
<th>Deflection</th>
<th>Between-subject factor</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>N20m, r.m.s. (fT)</td>
<td>Block</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>0.9</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Block $\times$ group</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td>P35m, r.m.s. (fT)</td>
<td>Block</td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>1.8</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Block $\times$ group</td>
<td>0.4</td>
<td>0.9</td>
</tr>
</tbody>
</table>

**Discussion**

In the present study, we examined the response properties of two distinct cortical generators in the SI following electrical stimulation of the median nerve in healthy subjects and patients with frequent migraine attacks during the interictal state of migraine. The cortical generators for N20m and P35m...
could be clearly separated by their different peak latencies as well as their opposing directions and the different source localization of their ECDs. These findings accord with previous reports (Wikström et al., 1996; Hoshiyama and Kakigi, 2001). Precise characterization of the activated cortical sources is the domain of MEG and has not been reported earlier in the context of migraine research. In this study, the exact differentiation of the two early SEF components disclosed differential changes in these components in migraineurs.

The main result was that the r.m.s. and ECD moment of N20m were significantly increased in the migraineurs included in this study and the magnitude of N20m was positively related to the frequency of migraine attacks. Additionally, there was a non-significant tendency for a correlation between the r.m.s. of N20m and the duration of the migraine attacks. In contrast, the r.m.s. of P35m in migraineurs did not differ from the values obtained from control subjects nor did it correlate with the frequency of migraine attacks. An increase in N20m in migraineurs suggests that the total number or the transmembrane current of the cortical neurons that are excited by each stimulus and that contribute to N20m is larger in migraineurs with frequent migraine attacks than in healthy subjects and hence indicates that there is a hyperexcitability of these SI neurons. Increased transmembrane current may result from a slightly more hyperpolarized membrane potential level in migraineurs. Based on the hypothetical model of Wikström et al. (1996), N20m represents an excitatory postsynaptic mass potential of the SI. Our finding may therefore indicate that excitatory SI neurons are hyperexcitable in the interictal state of migraine. In contrast, the excitability of the neural source of P35m which is hypothesized to represent inhibitory SI neurons was found to be in the physiological range of healthy controls.

Our results are in line with previous neurophysiological reports on cortical hyperexcitability in the interictal state of migraine, i.e. increased amplitudes of visual evoked potentials (Kennard et al., 1978; Diener et al., 1989; Shibata et al., 1997; Shibata et al., 1998) and contingent negative variation (Schoenen et al., 1985; Kropp and Gerber, 1993). Cortical hyperexcitability of the visual cortex is also suggested from psychophysical experiments showing a hypersensitivity of migraineurs in the interictal state to environmental light (Hay et al., 1994) and to grating patterns of definite spatial frequency (Wilkins et al., 1984; Marcus and Soso, 1989).

Lack of habituation of visual evoked potentials is considered to be an interictal phenotype marker in many migraineurs and a basic abnormality in cortical information processing (Ambrosini et al., 2003a). However, contrary to most previous studies, we could not find a significant habituation of N20m or P35m in the control subjects and therefore could not demonstrate an abnormal habituation process in the migraineurs. In healthy subjects, significant habituation has been reported for visual evoked potentials (Schoenen et al., 1996).
et al., 1995; Afra et al., 1998; Wang et al., 1999), somatosensory evoked potentials (Ozkul and Uckardes, 2002) and event-related potentials, such as the contingent negative variation (Schoenen et al., 1993; Kropp and Gerber, 1995) or the auditory novelty P3a in a passive oddball paradigm (Wang and Schoenen, 1998). The absence of habituation of N20m or P35m in the present study may be due to methodological causes, as for example the instruction for the subjects to keep their attention on the electrical stimuli high over the whole run. Sustained or repetitive attention delays habituation (Thompson and Spencer, 1966). Another cause for the apparent lack of habituation may be that the electrical stimulus at the stimulus intensities used was a novel experience for the subjects and could not be ignored until the end of the stimulation run. Although physiological habituation in the control subjects may be masked by the instruction to the subjects or the quality of the stimulus, the measured group differences support the hypothesis that the increased strength of N20m in migraineurs is due to cortical hyperexcitability.

R.m.s. values of N20m and P35m showed only small variations over the stimulation sequence. We preferred r.m.s. value as compared with ECD strength because values of ECD strength depend on the signal-to-noise ratio. For analysis of the present data, ECD strength could not be used as a reliable indicator for excitation of the cortical neurons in patients with a small r.m.s. value of N20m at an ISI of 0.3 s and in the averagings of small sweep numbers (increased noise) for analysis of habituation. In contrast, in the stimulation runs with ISIs between 0.5 and 6 s, the effects of group and age on the r.m.s. values and ECD strength of N20m and P35m were comparable.

In a previous study, a positive correlation between ECD strength of N20m and age (range 20–73 years) was reported (Huttunen et al., 1999). These data suggest a slight age-related increase in cortical excitability. In the present study, there was a non-significant tendency for correlation between age (range 20–62 years) and the r.m.s. value of N20m over all subjects, not within the groups of migraineurs and control subjects. However, there was no significant age effect on the r.m.s. values and ECD strength of N20m in the repeated measures ANOVA.

The selection of a patient group with a high migraine attack frequency has the associated risk of including patients with chronic migraine or drug-induced headache. Two patients had at least 15 days of migraine headache per month and therefore could be classified as chronic migraineurs according to the second edition of the International Classification of Headache Disorders (Kopfschmerzklassifikationskomitee der International Headache Society, 2003). Despite their intake of high doses of triptans, these patients did not report an increase of attack frequency with increase of triptan intake. Hence, they did not fulfill the criteria for triptan-induced headache. In addition, variation of the r.m.s. values in the subgroup of both patients does not support the idea that a possible cerebral mechanism leading to a chronic state of migraine had a major effect on the group results. This also seems to be unlikely for the total amount of triptans or analgesics consumed per month since this factor did not significantly correlate with the r.m.s. value of the N20m.

N20m is temporally linked to the N20 component of the somatosensory evoked potential after median nerve stimulation (Hoshiyama and Kakigi, 2001). In a somatosensory evoked potential study (Ozkul and Uckardes, 2002), the amplitude of N20 did not differ between migraineurs and healthy subjects. This result may be related to the lower frequency of migraine attacks (3.1 ± 0.3 attacks/month) of the patients in this study, indicating a less severe migraine disease. This explanation is supported by the result of the subgroup of patients with three and four migraine attacks/month in the present study. Another cause for the differences between the somatosensory evoked potential study and the present data may be that MEG cannot detect radially oriented currents and consequently it does not measure the activation of the same neuronal population as EEG. This explanation, however, could not be tested in the present study because we did not record EEG and MEG simultaneously.

In migraine, the response properties of the cortical generators of N20m and P35m during different ISIs are unchanged when compared with healthy subjects. Although the cause of the frequency-dependent depression of neuronal excitability is not known exactly (Creutzfeldt, 1995; Wikström et al., 1996), the results indicate that the possible mechanisms of cellular response refractoriness, such as a hyperpolarizing DC shift of the membrane potential (Hellweg et al., 1977) or an actual decrease in synaptic conductance (Deisz and Prince, 1989), are not altered in the SI neurons of migraineurs. Furthermore, the reported cellular abnormalities in migraineurs (Welch, 2003) such as genetically induced channelopathies (Estevez and Jardner, 2003) or mitochondrial dysfunction associated with abnormal energetic and metabolic reserves (Welch and Ramadan, 1995; Boska et al., 2002) seem not to be critically involved in the ISI-dependent depression of SI neurons.

N20m and P35m represent synchronized postsynaptic mass responses of distinct neuronal populations in the sensorimotor cortex elicited by afferent nerve stimulation. Our data indicate that a pathophysiological link exists between the r.m.s. of N20m and the cortical state in patients with high disease severity, i.e. frequent migraine attacks in the last 3 months. In contrast, the r.m.s. of N20m did not correlate significantly with the duration of the migraine disease. This result is in line with the clinical characteristics of our patients in whom the disease duration was not significantly related to the frequency of their migraine attacks. However, we do not know whether the r.m.s. of N20m in migraineurs varies with the disease severity over the patient’s lifetime or whether it instead represents a relatively stable biological marker of the subjects’ disposition to develop frequent migraine attacks. Therefore, we do not interpret a single measurement of N20m in migraineurs as a measure for a propensity towards a state of chronic migraine.

However, our results differ from those of a previous study in which a correlation between contingent negative variation
and disease duration was found (Kropp et al., 2000). The authors discussed this finding as a possible unspecific neurophysiological process of cortically represented chronicity. Differences between the two studies may be caused by differences in attack frequency in each class of disease duration. Furthermore, the short latency signal of the N20m and the contingent negative variation, a cognitively driven potential in the later time domains, may not be directly comparable.

It is known that the activity of cortical cells is controlled by the ‘ascending reticular activating system’. This system modulates the intensity of attention according to the general level of arousal and wakefulness (Mesulam, 1998). In the pathophysiology of migraine, the serotonergic projections from the raphe nuclei and the noradrenergic projections from the nucleus locus coeruleus to the cerebral cortex are of particular interest. Noradrenergic projections from the pontine nucleus locus coeruleus to the cortex increase the signal-to-noise ratio of afferent stimuli thereby improving the processing of significant sensory events (Foote et al., 1983; Levin et al., 1988). Increased N20m in migraineurs may result from abnormal functioning of these cortical ‘state setting’ (Mesulam, 1990) systems.

Pharmacological studies using the centrally acting acetylcholine antagonist scopolamine (Huttunen et al., 2001) showed that the N20m was not affected by central muscarinic blockade, while P35m was significantly reduced. However, the magnitude of N20m and P35m has not been examined by pharmacological modulation of the serotonergic and noradrenergic system. It would be of interest in further studies to evaluate whether for example β-blockers or serotonin re-uptake inhibitors decrease N20m in relation to the frequency of migraine attacks.

In conclusion, an increase in interictal r.m.s. and ECD strength of N20m indicates a hyperexcitability of the SI in patients with frequent migraine attacks. This hyperexcitability is linked to the frequency of migraine attacks. However, an increase in N20m cannot be explained by cortical disinhibition since habituation could not be found in the control subjects. In contrast, the r.m.s. of P35m is not related to migraine and this may indicate that there is a normal inhibitory cortical activation in response to afferent input in migraine patients. Furthermore, our findings suggest that there is no abnormality of the cellular mechanisms underlying the response properties of N20m and P35m to afferent stimuli with short ISIs in migraine patients.

References
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