EJN fens European Journal of Neuroscience

European Journal of Neuroscience, Vol. 30, pp. 714–720, 2009 doi:10.1111/j.1460-9568.2009.06859.x

COGNITIVE NEUROSCIENCE

Visual paired-pulse stimulation reveals enhanced visual cortex excitability in migraineurs

Oliver Höffken,[1] Philipp Stude,[1] Melanie Lenz,[1] Michael Bach,[2] Hubert R. Dinse[3] and Martin Tegenthoff[1]
[1]Department of Neurology, Ruhr-University Bochum, BG-Kliniken Bergmannsheil, Buerkle-de-la-Camp-Platz 1, 44789 Bochum, Germany
[2]Universitäts-Augenklinik Freiburg, Freiburg, Germany
[3]Department of Theoretical Biology, Institute for Neuroinformatics, Neural Plasticity Lab, Ruhr-University Bochum, Bochum, Germany

Keywords: electrophysiology, migraine, paired-pulse suppression, visual-evoked potentials

Abstract

Migraine is a common ictal disorder with an interindividual heterogeneous characteristic, whose underlying mechanisms remain elusive. On the one hand migraine is associated with abnormal cortical hyperexcitability. On the other hand, studies reported lower amplitudes of visual-evoked potentials (VEPs) and concluded that low preactivation levels imply decreased excitability. Here we measured visual cortex excitability and paired-pulse suppression in subjects suffering from migraine without aura and in a group of aged- and gender-matched healthy subjects to address the relation between activation levels and excitability. To that aim, we analysed amplitudes of VEPs and paired-pulse suppression evoked by a paired-pulse stimulation paradigm using stimulus onset asynchronies (SOAs) between 80 and 133 ms. We found that in migraineurs in the interictal state the amplitudes of the first VEP were reduced as compared with healthy subjects by approximately 20%. In the case of paired-pulse suppression comparable to healthy controls, the second response amplitude should be reduced as well, which was not the case. Instead, the ratio between the first and second VEP was higher than in healthy controls and did not depend on SOA in the range tested, which demonstrates reduced paired-pulse suppression and therefore implicates increased cortical excitability. Our data show that in migraineurs VEPs were reduced presumably due to reduced activation levels. However, paired-pulse suppression using short SOAs in the range of 100 ms or less was even higher than in normal subjects. Thus, our data show that signatures of both hyper- and hypoexcitability can be found depending on stimulation condition.

Introduction

Migraine is a common ictal disorder with an interindividual heterogeneous characteristic, whose underlying mechanisms are not completely understood. Next to genetic predisposition (Stam *et al.*, 2008) and influences of environmental factors, pathophysiological studies in patients with migraine and animal model of migraine headache have identified several involved neural structures like the trigeminovascular system, the brainstem (periaqueductal grey matter, aminergic nuclei) and the cerebral cortex (Bahra *et al.*, 2001; Goadsby, 2005). Due to the ictal character of migraine, the view focuses on the dynamics of neuronal and vascular components. An important pathophysiological role is attributed to the abnormal cortical excitability. However, studies with multimodal evoked potentials in motor, visual and somatosensory systems obtained under different interictal conditions provided controversial findings (Bohotin *et al.*, 2002; Ozkul & Uckardes, 2002; Schoenen *et al.*, 2003; Ambrosini & Schoenen, 2006; Huang *et al.*, 2006; Khedr *et al.*, 2006).

Correspondence: Dr O. Höffken, as above.
E-mail: oliver.hoeffken@ruhr-uni-bochum.de

Received 2 April 2009, revised 12 June 2009, accepted 22 June 2009

Various stimulation paradigms have been used to study visual-evoked responses in patients suffering from migraine to address effects of response amplitudes and to study differences between affected and not-affected hemispheres. Many lines of evidence suggest that the excitability of the visual cortex of patients suffering from migraine is altered. Studies using functional magnetic resonance imaging (Vincent *et al.*, 2003; Bramanti *et al.*, 2005), psycho-physical examinations (Palmer *et al.*, 2000; Mulleners *et al.*, 2001) and results of electrophysiological testing (Afra *et al.*, 1998; Gerwig *et al.*, 2005; Khedr *et al.*, 2006) of the visual cortex tried to shed light on the physiology of the underlying mechanisms.

The paired-pulse stimulation protocol, i.e. the application of two stimuli in close succession, allows investigating cortical excitability by measuring the suppressive effect of the second stimulus in comparison to the first. As a measure of paired-pulse suppression and thus of cortical excitability we use the amplitude ratio (A_2 /A_1) of the second and first response amplitudes ($C1_2 - C2_2$)/($C1_1 - C2_1$) (Fig. 1). Little paired-pulse suppression indicative of high cortical excitability is reported by a high-amplitude ratio, while small ratios point to large suppression indicative of reduced excitability.

Here we attempt to understand the divergent findings described above for patients with migraine by systematically studying the relation

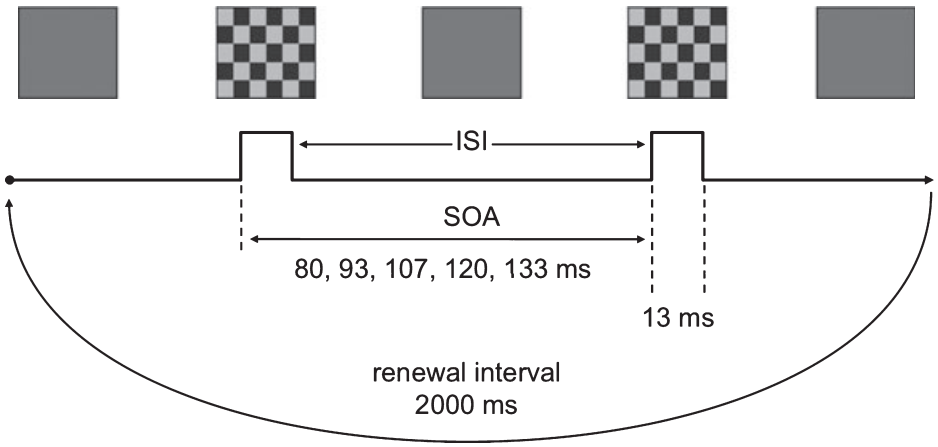

FIG. 1. The paired-pulse stimulation paradigm. The paired-pulse paradigm consists of checkerboard patterns, which were presented at different stimulus onset asynchronies [SOAs; SOA = interstimulus interval (ISI) + pulse duration]. The first stimulus appeared for one frame (13.33 ms), followed by presentations of frames containing a homogenous grey background without a change in the mean luminance. The second stimulus appeared after different SOAs (80 ms, 93 ms, 107 ms, 120 ms and 133 ms), each SOA presented 10 times followed by the next value. After the last SOA value the entire cycle repeated for a total of 4 circles (40 sweeps per SOA step).

between signal amplitudes and paired-pulse suppression, evoked by the new paired-pulse stimulation paradigm (Hoffken *et al.*, 2008), to measure visual cortex excitability and paired-pulse suppression. We used stimulus onset asynchronies (SOAs) between 80 and 133 ms of visual-evoked potentials (VEPs) in subjects suffering from migraine without aura and a group of aged- and gender-matched healthy controls.

Materials and methods

Thirty-eight subjects took part in the study: we tested 19 healthy control subjects without personal or family history of any primary headache and 19 outpatients (both with a mean age of 39 years, consisting of each 16 females and three males) with migraine without aura according to criteria of the ICHD-II classification (1994), with a history of migraine for at least 1 year. There are controversial findings concerning differences in excitability between migraineurs with and without aura (for an overview, see Afra *et al.*, 1998; Schoenen *et al.*, 2003). In order to form a homogenous group, we therefore restricted our sample to patients suffering from migraine without aura. The patients reported between one and three attacks per month (mean 2.11 ± 0.73 SEM), with a mean of 2.5 migraine days per month. None of them received a prophylactic anti-migraine treatment for at least 3 months before participating in the study. No subject suffered from any other neurological disease and all were free of any other regular medication, except of oral contraception in seven cases. The paired-pulse recordings were performed no sooner than 72 h after the last headache attacks. No patient experienced a headache attack following the recording within 72 h after participation in the study. In female subjects, the VEP recordings were done during both menstrual phases. Retrospective analysis showed that the time of examination was randomly distributed throughout the menstrual cycle. Before participation, all subjects gave their written informed consent. The study was approved by the Ethics Committee of the Ruhr-University Bochum and was performed in accordance with the Declaration of Helsinki.

Stimulation

The stimulation setting was the same as described in our previous study (Hoffken *et al.*, 2008). The stimuli were displayed on a CRT spanning 23° × 17° of visual angle at the observation distance of 80 cm. The CRT was set to a frame rate of 75 Hz and a pixel resolution of 800 × 600. The experimental paired-pulse paradigm consisted of checkerboard patterns with 36% contrast with a mean luminance of 16 cd/m^2, which were presented at different SOAs. The first stimulus appeared for one frame (13.33 ms), followed by presentations of frames containing a homogenous grey background without a change in the mean luminance (Fig. 1). The second stimulus appeared after variable SOAs in multiples of the frame interval of 13.33 ms to avoid temporal aliasing (Bach *et al.*, 1997). We used five different SOAs between 80 ms (5 frames) and 133 ms (9 frames) in which highest paired-pulse inhibition in healthy subjects was found (Hoffken *et al.*, 2008). Each SOA value was presented 10 times, and then the next value followed. After the last SOA value the entire cycle was repeated for a total of 40 sweeps per SOA step.

The stimuli were produced by the EP2000 system (Bach, 2000), which also recorded the electroencephalogram, and averaged and displayed the responses on-line. Gold-cup electrodes were attached to Oz and Cz (1994). Signals were amplified and filtered (1–100 Hz, 1st order band-pass) using a conventional Neuropack 8 equipment (Nihan Kohden), and digitized to 16 bit (resulting in 0.006 μV amplitude resolution) at 1 kHz sampling frequency in a Macintosh G4 computer running EP2000. Signals exceeding 140 μV were rejected as artefacts and not counted in the stimulation sequence.

Off-line, all traces were processed by a phase-neutral digital low-pass filter with a corner frequency of 40 Hz and trace features were interactively identified. We use the terms A1 and A2 to denote the amplitude of the response to the first and second stimulus. We use the term C to denote the positive and negative components of the responses (Fig. 2). To characterize the paired-pulse response, the amplitude difference of the $C1_1$ (a positivity before 100 ms after stimulus onset; Odom *et al.*, 2004) and the $C2_1$ (a negativity later than 100 ms after stimulus onset) was measured. The same measures ($C1_2$, $C2_2$) were obtained for the second pulse, and could unequivocally be identified for all SOAs. As a marker of paired-pulse suppression, the amplitude ratio $A2/A1 = C1_2 - C2_2/C1_1 - C2_1$ was calculated for all different SOAs.

Statistics

The data were analysed with a mixed effects (repeated-measures) ANOVA. Factors were SUBJECT, GROUP (control/migraine, within)

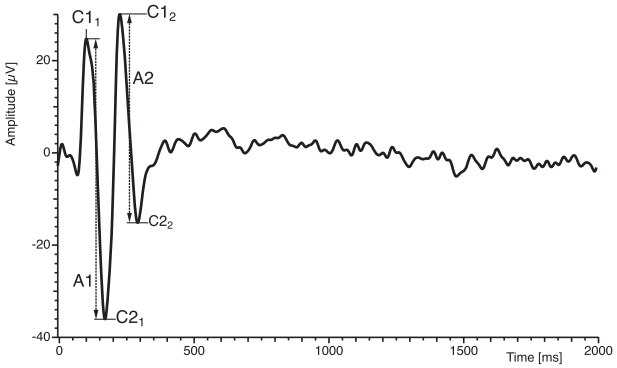

FIG. 2. VEP components in a paired-pulse paradigm. Shown are VEPs to paired-pulse stimulation at a SOA of 120 ms, $C1_1$ and $C2_1$ indicate the typical negative and positive onset-VEP peaks of the first response, $C1_2$ and $C2_2$ of the second response. Amplitude ratio = $A2/A1 = C1_2-C2_2/C1_1-C2_1$.

and SOA or A1 (first response) (five levels, within). Statistical calculations were performed in the R statistical system (R Development Core Team 2007) and SPSS 17.0 (Version 17.0.0) with subsequent sequential Bonferroni adjustment (Holm, 1979). Unpaired, two-tailed *t*-tests were used to analyse differences of gender and contraception use, and for *post hoc* analysis if the ANOVA revealed a significant interaction. For these *t*-tests, the significance level was adjusted by dividing it by the number of comparisons (0.05 /4 = 0.01; Bonferroni correction). Before using these parametric tests, normality was tested using the Kolmogorov–Smirnov test, and homogeneity of variances was confirmed by a Levene test. Additionally, Pearson's correlation coefficient was calculated in order to detect any possible relationship between paired-pulse suppression and different clinical parameters. Significance was assumed at the $P = 0.05$ level.

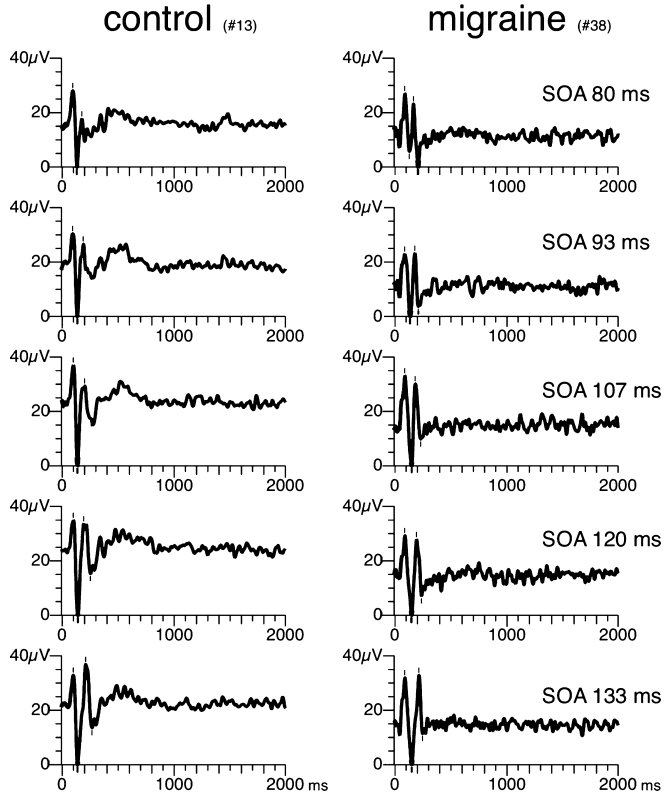

FIG. 3. Cortical VEP-responses to paired-pulse stimulation of one subject of each group (control left, migraine right) for all stimulus onset asynchronies (SOAs) tested, positivity upwards. For brief SOAs (top), the second stimulus evoked a smaller response, for large SOAs less suppression is found (bottom).

Procedure

During the recording sessions subjects sat in a comfortable chair in a darkened room at a distance of 80 cm from the stimulus screen. Two electrodes (Oz and Cz) were positioned according to the International 10-20-system. A reference electrode was placed over the Fpz-position. Subjects were instructed to relax and to keep their eyes focussed on the centre of the display marked by a small dim cross, which was displayed during the entire course of the measurements. The testing paradigm consisted of one session with five different SOAs.

Results

Recording VEPs to paired-pattern-pulse stimulation at SOAs between 80 and 133 ms elicited clearly distinguishable responses, where the second response component was suppressed to varying extents (Fig. 3). For further analysis, we calculated the amplitude ratios of the cortical evoked responses to paired-pattern-pulse stimulation with respect to the SOA. Particularly in the healthy control group the ratios increased from low values at short SOAs, indicating strong paired-pulse suppression, to high values observed at longer SOAs indicative of less suppression. For the subjects of the migraine group this effect was less clear. The results from the Kolmogorov-Smirnov test showed that the amplitude-ratios had a normal cumulative distribution function (SOA of 80 ms $P = 0.977$, KS = 0.477; SOA of 93 ms $P = 0.539$, KS = 0.803; SOA of 107 ms $P = 876$, KS = 0.591; SOA of 120 ms $P = 0.39$, KS = 0.902; SOA of 133 ms $P = 0.593$, KS = 0.771). The influence of SOA on the paired-pulse ratio of both groups by a one-way ANOVA for repeated measurements (within-subject factor SOA and between-subject factor amplitude ratio) yielded a highly significant effect with $P < 0.0001$ at $F_{4,144} = 6.048$. According to ANOVA, for the amplitude ratio (A2/A1) there were significant effects of group (control vs. migraine; $P = 0.027$, $F_{1,36} = 5.297$), of SOA ($P < 0.0001$, $F_{4,144} = 6.048$), and of interaction of SOA and group ($P = 0.02$, $F_{4,144} = 3.003$). *Post hoc* analysis with two-tailed, unpaired, Student's *t*-test showed significantly lower paired-pulse suppression at SOAs of 80 ms ($P = 0.005$) and 93 ms ($P = 0.001$) in the migraine group compared with the control group.

In line with the ANOVA results, Fig. 4a illustrates that the amplitude ratios in the migraine group do not depend on SOA, but rise monotonously with increasing SOA in the control group (indicated by the significant interaction term group and SOA).

The magnitude of paired-pulse suppression is a marker of cortical excitability. Under the assumption that abnormal cortical excitability plays an important pathophysiological role in patients suffering from migraine, we performed a linear correlation analysis between the extent of paired-pulse suppression, which can be observed at short SOAs (80 and 93 ms), and the frequency of migraine attacks and the number of days with migraine. While there was no relation between paired-pulse suppression and number of days with migraine, we observed a significant correlation between suppression and the frequency of migraine attacks (Table 1).

To address potential gender-specificity, we analysed paired-pulse suppression observed at all measured SOAs separately for female and male participants in both groups, which revealed no differences in two-tailed, unpaired, Student's *t*-test. Furthermore, a linear correlation

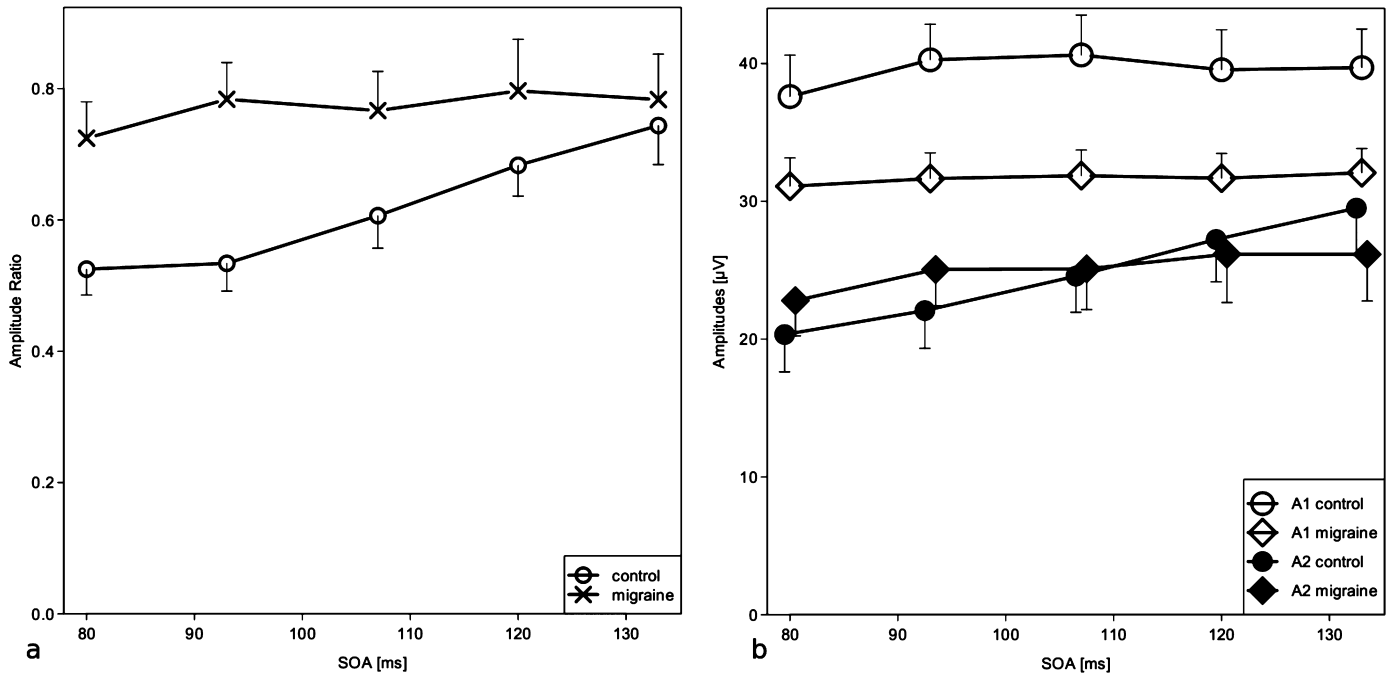

FIG. 4. (a) Amplitude ratios of the migraine and control group as a function of stimulus onset asynchronies (SOAs), grand mean ± SEM of all 19 subjects. (b) Amplitudes in response to the first stimulus (A1, open symbols, top) and in response to the second stimulus (A2, filled symbols, below) for the healthy controls (circles) and the migraine group (squares) vs. SOA. The data points represent the mean, the error bars SEM.

TABLE 1. Linear correlation analysis*

	Frequency of migraine attacks	
SOA	*r*-value	*P*-value
80 ms	0.335	0.161
93 ms	0.5	0.029

*Pearson (*r*) and *t*-test (*P*) between frequency of migraine attacks and paired-pulse suppression at SOAs of 80 and 93 ms in the migraine group. SOA, stimulus onset asynchrony.

analysis revealed no significant relation between the degree of paired-pulse suppression and the age of the subjects in both groups (at SOA of 80 ms: $r = 0.285$, $P = 0.237$; SOA of 93 ms: $r = 0.344$, $P = 0.15$). In the migraine group, seven of the 16 female patients used oral contraception. To determine possible effects of oral contraception on paired-pulse suppression, we analysed paired-pulse suppression in the migraine group. We found no significant differences of amplitude ratios in the measured SOA (SOA 80 ms $P = 0.512$, SOA 93 ms $P = 0.322$, SOA 107 ms $P = 0.856$, SOA 120 ms $P = 0.753$, SOA 133 ms $P = 0.833$).

In principle, an increased amplitude ratio and thus a reduced paired-pulse suppression as found in the migraine group as compared with the controls can be achieved by two different types of alterations of the response behaviour: either by an increase of the second response; or by a reduction of the first response. We therefore analysed separately A1 and A2. The results from the K-S test find no significant values for the amplitudes of the first and second response, indicating that the distribution is normal.

Regarding the first VEP amplitude (A1), in the migraine group A1 was significantly smaller reaching only 80.1% of the controls (ANOVA control vs. migraine; $P = 0.019$, $F_{1,36} = 5.994$). Regarding the second VEP amplitude (A2), for both groups we found that A2 was always

TABLE 2. Response amplitudes and their ratios for the the migraine group and controls

	SOA (ms)				
Parameter	80	93	107	120	133
First amplitude (A1, µV)					
Control group	37.60 ± 3.01	40.26 ± 2.59	40.62 ± 2.89	39.54 ± 2.90	39.76 ± 2.87
Migraine	31.09 ± 2.01	31.65 ± 1.81	31.86 ± 1.82	31.67 ± 1.75	32.07 ± 1.71
Second amplitude (A2, µV)					
Control group	20.90 ± 2.72	22.77 ± 2.72	25.25 ± 2.60	27.83 ± 3.10	30.08 ± 3.37
Migraine	22.79 ± 2.50	25.05 ± 2.56	25.10 ± 2.88	26.16 ± 3.42	26.15 ± 3.29
Amplitude ratio (A2/A1)					
Control group	0.54 ± 0.04	0.55 ± 0.04	0.62 ± 0.05	0.70 ± 0.05	0.76 ± 0.06
Migraine group	0.72 ± 0.06	0.78 ± 0.06	0.77 ± 0.06	0.80 ± 0.07	0.78 ± 0.07

Data are presented as mean values (grand means) ± SEM of first and second response and amplitude ratio for the healthy control and the migraine group. SOA, stimulus onset synchrony.

TABLE 3. *Post hoc* unpaired *t*-tests for group difference (migraine and control)

	SOA (ms)				
	80	93	107	120	133
Amplitude ratio	0.005*	0.001*	0.043	0.205	0.651
First amplitudes	0.075	0.009*	0.013	0.024	0.024

SOA, stimulus onset asynchrony. *$P < 0.01$.

lower than A1 (control group: $P < 0.0001$; $F_{1,36} = 14.94$; migraine group: $P = 0.031$; $F_{1,36} = 5.095$). However, while A2 increased with increasing SOA for the control group ($P < 0.0001$; $F_{4,72} = 17.694$), A2 remained constant in the migraine group ($P = 0.221$; $F_{4,72} = 1.468$) (Table 2). ANOVA revealed no significant effect of group (control vs. migraine; $P = 0.937$, $F_{1,36} = 0.006$), but a significant effect of SOA ($P < 0.0001$, $F_{4,144} = 11.968$), and a significant interaction of group and SOA ($P = 0.013$, $F_{4,144} = 3.29$).

The significant effect of group for A1 indicates that there is a significant reduction in VEP amplitude in the migraine group compared with the controls. The significant effect of SOA, in combination with the significant interaction, fits with the data shown in Fig. 4b, where in the migraine group A2 is little affected by SOA (nearly a horizontal line), whereas in the control group there is a monotonous increase of A2 with SOA (Table 3). According to our data the amplitude of the response to the first stimulus plays a crucial role in controlling paired-pulse behaviour in migraineurs.

Discussion

The aim of this study was to assess paired-pulse behaviour as a marker of cortical excitability of the visual cortex in patients suffering from migraine, and compare these data to healthy controls using a new paired-pulse stimulation protocol to record VEPs (Hoffken *et al.*, 2008).

We found significantly reduced paired-pulse suppression in the visual cortex in migraineurs compared with a healthy control group, as indicated by an enhanced amplitude ratio (A2/A1 - quotient of the second and the first response amplitude in the migraine group). Further analysis revealed that the magnitude of the first response component was significantly smaller in patients with migraine. In the case of unchanged paired-pulse suppression, the second response amplitude should be reduced as well, which was, however, not the case. Instead, the ratio between first and second response was higher than in healthy controls, indicative of reduced paired-pulse suppression in migraineurs most likely caused by a migraine-induced reduction of the first response amplitude. The fact that the amplitude to the first stimulus did not depend on SOA indicates that the spacing between trials, i.e. the renewal interval of 2 s, was sufficient.

The interictal alteration of cortical excitability in patients suffering from migraine has been investigated in numerous electrophysiological studies. In the current literature there is controversy about the character of alterations, for which different terms are used by different groups, such as excitability, preactivation level, habituation, gating, hyperresponsitivity, hypersensitivity, hyperreactivity or cortical dysbalance (Coppola *et al.*, 2007).

We here use the term paired-pulse behaviour to describe the overall response dependence on different SOAs. We use the term (paired-pulse)-suppression to refer to the reduction of the neuronal response to the second stimulus, a phenomenon often denoted as forward suppression or short-term plasticity (Zucker & Regehr, 2002). We will use inhibition to refer to one possible candidate for this suppression, namely GABAergic intracortical inhibition. By synaptic depression we refer to a reduction in synaptic drive. Mechanisms mediating synaptic depression include postsynaptic receptor desensitization, presynaptic depletion of releasable vesicles or other presynaptic mechanisms (Bellingham & Walmsley, 1999). Neural refractoriness may play a role only at very short interstimulus intervals (ISIs; Hoshiyama & Kakigi, 2003). In addition, the term excitability is used to characterize the sensitivity of a neural system. Specifically, the neural response behaviour after single stimulus presentation is often described in terms of preactivation. Finally, habituation is defined as a response decrement as a result of repeated stimulations and its lack is supposed to be an essential pathophysiological feature of the migraine's disorder.

In the past, VEPs in patients suffering from migraine have been studied with various stimulation paradigms. With few exceptions (Richey *et al.*, 1966), early studies using single, flash-evoked visual potentials observed higher VEP amplitudes in migraineurs compared with controls (Lehtonen, 1974; Connolly *et al.*, 1982; for overview, see Schoenen *et al.*, 2003). More recently, pattern-reversal VEPs were employed, which revealed increased VEP amplitudes (Shibata *et al.*, 1997; Khalil *et al.*, 2000), decreased amplitudes (Polich *et al.*, 1986; Tagliati *et al.*, 1995), but in most cases no differences between migraineurs and healthy controls (Sener *et al.*, 1997; Sand & Vingen, 2000).

To overcome the problems associated with assessing excitability from single-stimulus presentations, during the last years paired-pulse stimulation has become a common tool to investigate cortical excitability to obtain insight into contributions of intracortical inhibition and facilitation, and changes in the balance of both (Kujirai *et al.*, 1993; Ziemann *et al.*, 1996).

In order to obtain a measure of paired-pulse suppression and thus of cortical excitability in patients with migraine we here applied a recently developed paired-pulse paradigm where checkerboard patterns appeared interleaved with a homogenous grey background without a change in the mean luminance (Hoffken *et al.*, 2008). We found that migraineurs show reduced paired-pulse suppression of the second VEP-amplitude at short SOAs, indicating enhanced intracortical excitability despite reduced first VEP amplitudes. Paired-pulse behaviour was investigated in different sensory systems to explore the altered cortical mechanism in migraineurs. In the auditory system, Ambrosini *et al.* (2001) found significantly less reduced amplitudes to a second auditory-evoked response of two homologues stimuli in migraineurs compared with healthy volunteers. According to the authors' interpretation, this finding might be due to a reduced sensory gating as a hypofunction of monoaminergic subcortico-cortical pathways.

To explore a marker of excitability in somatosensory system, Valeriani *et al.* (2005) studied somatosensory-evoked potentials after paired-pulse stimulation at different ISIs in children suffering from migraine. Compared with a healthy control group the amplitudes of the cortical N20, P24 and N30 components at ISIs of 20 and 40 ms showed a significantly better recovery. Accordingly, they concluded that paediatric migraine is characterized by the development of intracortical disinhibition, which is in line with the hypothesis of a somatosensory cortex hyperexcitability.

Besides the influence of migraine, other conditions such as learning, environmental enrichment or brain injury affect paired-pulse behaviour, however, in quite specific ways. In particular, modulation of the first and second response amplitudes has been shown to be differentially involved. For example, in rats that were reared in an enriched environment the response to the first tone was increased, while the second remained unaffected, thereby enhancing the degree of paired-pulse suppression (Percaccio *et al.*, 2005). In humans, after perceptual

learning induced by passive stimulation (Pleger *et al.*, 2001, 2003; Dinse *et al.*, 2003), reduced paired-pulse suppression was observed where the individual gain in perceptual performance correlated with the reduction in suppression (Hoffken *et al.*, 2007). In this case, the response magnitude of the first stimulus remained unaffected, while the coactivation-induced suppression was due to an increased response to the second stimulus. Finally, damage of cortical tissue is known to lead to hyperexcitability, which dramatically alters paired-pulse behaviour (Schmidt *et al.*, 2006). In rats with hypoxic ischaemic brain injury, the magnitude of the first peak remained unaffected despite hyperexcitability, while the response to the second stimulus was substantially enhanced resulting in reduced suppression (Geissler *et al.*, 2007).

Taken together, paired-pulse suppression can be altered in at least two qualitatively different ways, namely by either changing the response to the first stimulus, or by changing the response magnitude of the second stimulus. This implies that the alterations of paired-pulse behaviour measured after cortical lesions or tactile coactivation are controlled by other mechanisms than those involved in changes induced by migraine. Our findings of reduced first VEP amplitudes in migraineurs may be explained by a lowered preactivation level in the visual cortex. It has been argued that a low preactivation level would allow a wide range of suprathreshold activation before reaching the 'ceiling' and initiating a 'reducing' response (Schoenen *et al.*, 2003). The preactivation level of cortical excitability seems to depend on 'state-setting, chemically addressed connections' that originate in the brainstem and involve serotonin and noradrenaline as transmitters (Schoenen *et al.*, 2003).

Despite substantial experimental and theoretical work, the mechanisms mediating paired-pulse behaviour are not fully understood. As discussed above, development of hyperexcitability as derived from reduced paired-pulse suppression not necessarily translates into changes of first response amplitudes. Conceivably, changes in paired-pulse suppression might reflect changes in intracortical processing, while the observation of reduced first amplitudes most likely reflects an involvement of thalamocortical transmission. Contradicting results might also arise from semantic confounds related to the term excitability. In any event, the development of altered response amplitudes parallel to changes of paired-pulse suppression in migraineurs might indicate regulatory deficits involving intracortical, feedforward and recurrent networks.

We conclude that in patients with migraine the cortical preactivation level is reduced as part of a compensatory, protective mechanism against increased excitability and overstimulation by external stimuli. Our data are in accord with the hypothesis of a reduction of response magnitudes to visual stimulation presumably due to changes of preactivation. However, the amount of paired-pulse suppression when using short SOAs in the range of 100 ms or less is even higher than in normal subjects. These results show that visual cortex activation in migraineurs depends on the type and timing of stimuli, and may thus provide an explanation as to why signatures of hyperexcitability are found in some studies, but not in others.

Acknowledgements

This work was supported by a grant from 'Forschungsförderung Ruhr-Universität Bochum Medizinische Fakultät (FoRUM)'.

Abbreviations

SOA, stimulus onset asynchrony; VEP, visual-evoked potential.

References

Afra, J., Mascia, A., Gerard, P., Maertens de Noordhout, A. & Schoenen, J. (1998) Interictal cortical excitability in migraine: a study using transcranial magnetic stimulation of motor and visual cortices. *Ann. Neurol.*, **44**, 209–215.

Ambrosini, A. & Schoenen, J. (2006) Electrophysiological response patterns of primary sensory cortices in migraine. *J. Headache Pain*, **7**, 377–388.

Ambrosini, A., De Pasqua, V., Afra, J., Sandor, P.S. & Schoenen, J. (2001) Reduced gating of middle-latency auditory evoked potentials (P50) in migraine patients: another indication of abnormal sensory processing? *Neurosci. Lett.*, **306**, 132–134.

Bach, M. (2000) *Freiburg evoked potentials*. Accessed 19 September 2007 (http://www.michaelbach.de/ep2000.html).

Bach, M., Meigen, T. & Strasburger, H. (1997) Raster-scan cathode-ray tubes for vision research – limits of resolution in space, time and intensity, and some solutions. *Spat. Vis.*, **10**, 403–414.

Bahra, A., Matharu, M.S., Buchel, C., Frackowiak, R.S. & Goadsby, P.J. (2001) Brainstem activation specific to migraine headache. *Lancet*, **357**, 1016–1017.

Bellingham, M.C. & Walmsley, B. (1999) A novel presynaptic inhibitory mechanism underlies paired pulse depression at a fast central synapse. *Neuron*, **23**, 159–170.

Bohotin, V., Fumal, A., Vandenheede, M., Gerard, P., Bohotin, C., Maertens de Noordhout, A. & Schoenen, J. (2002) Effects of repetitive transcranial magnetic stimulation on visual evoked potentials in migraine. *Brain*, **125**, 912–922.

Bramanti, P., Grugno, R., Vitetta, A., Di Bella, P., Muscara, N. & Nappi, G. (2005) Migraine with and without aura: electrophysiological and functional neuroimaging evidence. *Funct. Neurol.*, **20**, 29–32.

Connolly, J.F., Gawel, M. & Rose, F.C. (1982) Migraine patients exhibit abnormalities in the visual evoked potential. *J. Neurol. Neurosurg. Psychiatry*, **45**, 464–467.

Coppola, G., Pierelli, F. & Schoenen, J. (2007) Is the cerebral cortex hyperexcitable or hyperresponsive in migraine? *Cephalalgia*, **27**, 1427–1439.

Dinse, H.R., Ragert, P., Pleger, B., Schwenkreis, P. & Tegenthoff, M. (2003) Pharmacological modulation of perceptual learning and associated cortical reorganization. *Science*, **301**, 91–94.

Geissler, M., Neuhoff, S., Kreikemeier, K., Meier, C. & Dinse, H.R. (2007) Human umbilical cord blood cells restore cortical maps and cortical excitability after hypoxic ischemia in rats. Soc. Neurosci. Abstr. Abstr. Neuroscience Meeting Planner. Society for Neuroscience. Online, pp. 899.814/W897.

Gerwig, M., Niehaus, L., Kastrup, O., Stude, P. & Diener, H.C. (2005) Visual cortex excitability in migraine evaluated by single and paired magnetic stimuli. *Headache*, **45**, 1394–1399.

Goadsby, P.J. (2005) Migraine pathophysiology. *Headache*, **45**(Suppl 1), S14–S24.

Hoffken, O., Veit, M., Knossalla, F., Lissek, S., Bliem, B., Ragert, P., Dinse, H.R. & Tegenthoff, M. (2007) Sustained increase of somatosensory cortex excitability by tactile coactivation studied by paired median nerve stimulation in humans correlates with perceptual gain. *J. Physiol.*, **584**, 463–471.

Hoffken, O., Grehl, T., Dinse, H.R., Tegenthoff, M. & Bach, M. (2008) Paired-pulse behavior of visually evoked potentials recorded in human visual cortex using patterned paired-pulse stimulation. *Exp. Brain Res.*, **188**, 427–435.

Holm, S. (1979) A simple sequentially rejective multiple test procedure. *Scand. J. Stat.*, **6**, 65–70.

Hoshiyama, M. & Kakigi, R. (2003) Changes in somatosensory evoked responses by repetition of the median nerve stimulation. *Clin. Neurophysiol.*, **114**, 2251–2257.

Huang, J., DeLano, M. & Cao, Y. (2006) Visual cortical inhibitory function in migraine is not generally impaired: evidence from a combined psychophysical test with an fMRI study. *Cephalalgia*, **26**, 554–560.

ICHD-II classification (1994) Guideline thirteen: guidelines for standard electrode position nomenclature. American Electroencephalographic Society. *J. Clin Neurophysiol.*, **11**, 111–113.

Khalil, N.M., Legg, N.J. & Anderson, D.J. (2000) Long term decline of P100 amplitude in migraine with aura. *J. Neurol. Neurosurg. Psychiatry*, **69**, 507–511.

Khedr, E.M., Ahmed, M.A. & Mohamed, K.A. (2006) Motor and visual cortical excitability in migraineurs patients with or without aura: transcranial magnetic stimulation. *Neurophysiol. Clin.*, **36**, 13–18.

Kujirai, T., Caramia, M.D., Rothwell, J.C., Day, B.L., Thompson, P.D., Ferbert, A., Wroe, S., Asselman, P. & Marsden, C.D. (1993) Corticocortical inhibition in human motor cortex. *J. Physiol.*, **471**, 501–519.

Lehtonen, J.B. (1974) Visual evoked cortical potentials for single flashes and flickering light in migraine. *Headache*, **14**, 1–12.

Mulleners, W.M., Aurora, S.K., Chronicle, E.P., Stewart, R., Gopal, S. & Koehler, P.J. (2001) Self-reported photophobic symptoms in migraineurs and controls are reliable and predict diagnostic category accurately. *Headache*, **41**, 31–39.

Odom, J.V., Bach, M., Barber, C., Brigell, M., Marmor, M.F., Tormene, A.P., Holder, G.E. & Vaegan (2004) Visual evoked potentials standard (2004). *Doc. Ophthalmol.*, **108**, 115–123.

Ozkul, Y. & Uckardes, A. (2002) Median nerve somatosensory evoked potentials in migraine. *Eur. J. Neurol.*, **9**, 227–232.

Palmer, J.E., Chronicle, E.P., Rolan, P. & Mulleners, W.M. (2000) Cortical hyperexcitability is cortical under-inhibition: evidence from a novel functional test of migraine patients. *Cephalalgia*, **20**, 525–532.

Percaccio, C.R., Engineer, N.D., Pruette, A.L., Pandya, P.K., Moucha, R., Rathbun, D.L. & Kilgard, M.P. (2005) Environmental enrichment increases paired-pulse depression in rat auditory cortex. *J. Neurophysiol.*, **94**, 3590–3600.

Pleger, B., Dinse, H.R., Ragert, P., Schwenkreis, P., Malin, J.P. & Tegenthoff, M. (2001) Shifts in cortical representations predict human discrimination improvement. *Proc. Natl Acad. Sci. USA*, **98**, 12255–12260.

Pleger, B., Foerster, A.F., Ragert, P., Dinse, H.R., Schwenkreis, P., Malin, J.P., Nicolas, V. & Tegenthoff, M. (2003) Functional imaging of perceptual learning in human primary and secondary somatosensory cortex. *Neuron*, **40**, 643–653.

Polich, J., Ehlers, C.L. & Dalessio, D.J. (1986) Pattern-shift visual evoked responses and EEG in migraine. *Headache*, **26**, 451–456.

R Development Core Team (2007) *R: A language and environment for statistical computing*. Accessed 31 July 2008, from (http://www.R-project.org).

Richey, E.T., Kooi, K.A. & Waggoner, R.W. (1966) Visually evoked responses in migraine. *Electroencephalogr. Clin. Neurophysiol.*, **21**, 23–27.

Sand, T. & Vingen, J.V. (2000) Visual, long-latency auditory and brainstem auditory evoked potentials in migraine: relation to pattern size, stimulus intensity, sound and light discomfort thresholds and pre-attack state. *Cephalalgia*, **20**, 804–820.

Schmidt, S., Bruehl, C., Hagemann, G., Witte, O.W. & Redecker, C. (2006) Impairment of functional inhibition in the contralateral cortex following perinatally acquired malformations in rats. *Exp. Neurol.*, **201**, 270–274.

Schoenen, J., Ambrosini, A., Sandor, P.S. & Maertens de Noordhout, A. (2003) Evoked potentials and transcranial magnetic stimulation in migraine: published data and viewpoint on their pathophysiologic significance. *Clin. Neurophysiol.*, **114**, 955–972.

Sener, H.O., Haktanir, I. & Demirci, S. (1997) Pattern-reversal visual evoked potentials in migraineurs with or without visual aura. *Headache*, **37**, 449–451.

Shibata, K., Osawa, M. & Iwata, M. (1997) Pattern reversal visual evoked potentials in classic and common migraine. *J. Neurol. Sci.*, **145**, 177–181.

Stam, A.H., van den Maagdenberg, A.M., Haan, J., Terwindt, G.M. & Ferrari, M.D. (2008) Genetics of migraine: an update with special attention to genetic comorbidity. *Curr. Opin. Neurol.*, **21**, 288–293.

Tagliati, M., Sabbadini, M., Bernardi, G. & Silvestrini, M. (1995) Multichannel visual evoked potentials in migraine. *Electroencephalogr. Clin. Neurophysiol.*, **96**, 1–5.

Valeriani, M., Rinalduzzi, S. & Vigevano, F. (2005) Multilevel somatosensory system disinhibition in children with migraine. *Pain*, **118**, 137–144.

Vincent, M., Pedra, E., Mourao-Miranda, J., Bramati, I.E., Henrique, A.R. & Moll, J. (2003) Enhanced interictal responsiveness of the migraineous visual cortex to incongruent bar stimulation: a functional MRI visual activation study. *Cephalalgia*, **23**, 860–868.

Ziemann, U., Rothwell, J.C. & Ridding, M.C. (1996) Interaction between intracortical inhibition and facilitation in human motor cortex. *J. Physiol.*, **496**(Pt 3), 873–881.

Zucker, R.S. & Regehr, W.G. (2002) Short-term synaptic plasticity. *Annu. Rev. Physiol.*, **64**, 355–405.