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Phosphene thresholds correlate with paired-pulse suppression of visually evoked potentials

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ABSTRACT

Background: Phosphene thresholds (PT) induced by transcranial magnetic stimulation (TMS) as well as paired-pulse suppression (PPS) of visually evoked potentials (VEP) are used to characterize visual cortex excitability, however, their relation remains unknown.
Methods: We measured PT after single TMS over the occipital lobe, and recorded VEPs after paired-pulse stimulation at short stimulus-onset-asynchronies in the same subject. PPS was expressed by the ratio second to first response.
Results: We found a negative correlation between PT and PPS ($r = -0.36$, $P = 0.039$) indicating that higher PT were associated with smaller ratios indicative of low excitability, and vice versa. There was no difference in PPS between subjects who perceived phosphenes and those who did not.
Conclusions: Although both approaches target different mechanisms, PT and PPS seem to reflect common characteristics of visual cortex excitability. The lack of differences in PPS in subjects not perceiving phosphenes suggests that they might not have higher excitability levels.

Introduction

Transcranial magnetic stimulation over the occipital lobe can induce a brief perception of light sensations in the visual field, so-called phosphenes. Thresholds to induce phosphene perception expressed as relative intensities of maximal stimulator outputs are a common tool to measure excitability in human visual cortex. By using phosphene thresholds, altered excitability in visual cortex was found in patients suffering from migraine [1,2], ecstasy users [3], healthy subjects with photosensitivity or photoparoxysmal response [4], after appliance of transcranial direct current stimulation [5], after medical treatment with anticonvulsants [6,7] and light deprivation [8,9]. The examination of TMS-induced phosphenes is the most common used instrument to explore excitability in visual cortex. However, the detection and evaluation includes subjective factors and depends on the compliance of the respective subject. Furthermore, some subjects fail to perceive phosphenes [10,11]. In recent studies, we investigated paired-pulse VEP behaviour in order to obtain an alternative approach to explore excitability of visual cortex [12,13]. In analogy to paired-pulse paradigms in motor and somatosensory system [14–17], paired-pulse visually evoked potentials (VEP) provide information about paired-pulse suppression (PPS), an indirect marker of cortical excitability, which is used to characterize plastic changes in visual cortex [12,18–21]. We recently reported an enhanced excitability of visual cortex in patients suffering from migraine [13], which is in line with observations about reduced phosphene thresholds in these patients.

The aim of the present study was to investigate a possible relation between TMS-induced phosphene thresholds and VEP amplitude-ratios after paired-pulse stimulation assessed in the same individual.

Methods

Subjects

50 healthy subjects (25.0 ± 4.0 years [mean ± SD]; 25 females and 25 males) participated in this study. All subjects were free from any regular medication and from neurological diseases. All subjects gave their 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.

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Phosphene thresholds

Subjects were seated in a semi-darkened room with their head fixed on a chin rest. We administered single-pulse TMS, using the Magstim stimulator (Magstim, Whitland, Dyfed, U.K.) with a figure-of-eight shaped coil (outside diameter 8.7 cm, peak magnetic field strength 2.2 T, peak electric field strength 660 V/m). The coil was fixed on a tripod and the handle was orientated upwards. The coil was placed in the midline 1–5 cm above inion. The subjects were stimulated with supposed suprathreshold intensity up to 100% of maximal stimulator output until a phosphene was perceived. To determine an optimal position to induce phosphenes the coil was shifted in a horizontal line in 1 cm steps to both sides, and if necessary in parallel lines 1 cm above or below. To determine phosphene thresholds at this position, we applied single-pulse TMS in an interval of about 10 s with increasing stimulator outputs starting with 30% in 5% steps until phosphenes were reported. Then we proceeded in 1% steps in a randomized order above or below the supposed threshold. In analogy to previous studies, phosphene threshold was defined as the minimum stimulus intensity of stimulator output to induce phosphenes in three out of five trials [3,7,11].

Paired-pulse stimulation

During the VEP recording in a semi-darkened room, the subjects sat in a comfortable chair in front of a stimulation screen (cathode ray tube, frame rate 75 Hz, pixel resolution 800 × 600, spanning 23° × 17° of visual angle at the observation distance of 60 cm). 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 focused on the centre of the display marked by a small dim cross, which was displayed during the entire course of the measurements.

The experimental paired-pulse paradigm consisted of checkerboard patterns with 36% contrast and a check size of 0.5° with a mean luminance of 16 cd/m^2, which were presented at two different stimulus-onset-asynchronies (SOA). 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. To avoid temporal aliasing, the second stimulus appeared after variable SOAs in multiples of the frame interval of 13.33 ms [22]. We used two different SOAs of 107 ms (7 frames) and of 133 ms (9 frames), which in recent studies had revealed paired-pulse inhibition [12,13]. The SOAs were presented interleaved in 4 cycles of 10 paired stimuli each (each SOA was presented 40 times overall; stimulation frequency was 1 Hz). The interstimulus periods consists of a homogenous grey background while the mean luminance was kept constant. In another session, single visual evoked potentials with a sequence of 100 checkerboard patterns, at the same contrast and luminance used in the paired-pulse paradigm were presented for one frame (13.33 ms) followed by frames containing a homogenous grey background (intertrial interval 1000 ms; resulting stimulation frequency about 1 Hz) without a change in the mean luminance. The stimuli were produced by the EP2000 system [23]. VEPs were recorded and stored for offline analysis with a 32-channel-amplifier (Brain Amp, Brain Products, Germany, sampling rate 5 kHz, band-pass filtering between 2 and 1000 Hz). Evoked potentials after single and paired-pulse stimulation were recorded in epochs from 200 ms before and 400 ms after the stimulus, baseline corrected to the pre-stimulus interval and averaged. Signals exceeding 140 μV were rejected as artifacts and not counted in the stimulation sequence. 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. 1). To characterize the paired-pulse response, the amplitude difference of the C1 (a positivity before 100 ms after stimulus onset [24]) and the C2 (a negativity later than 100 ms after stimulus onset) was measured. To assess the paired-pulse interaction, confounds from superposition were removed by subtracting the response to a single pulse stimulation from the paired-pulse stimulation trace. We analyzed the amplitude of the response to the second stimulus of the paired-pulse stimulation after subtraction of the response to single pulse stimulation (second amplitude after subtraction = A2s) and referred it to the response to the first stimulus of the paired-pulse stimulation before subtraction (A1). Paired-pulse suppression was expressed as a ratio (A2s/A1) of the amplitudes of the second (A2s) and the first (A1) peaks (see Fig. 1). The stimulation setup and recording procedure was in analogy to the described procedure in our previous studies [12,13]. Assuming a common basic mechanism of paired-pulse inhibition at the SOAs used, we averaged the analyzed amplitude-ratios of both SOAs for each subject.

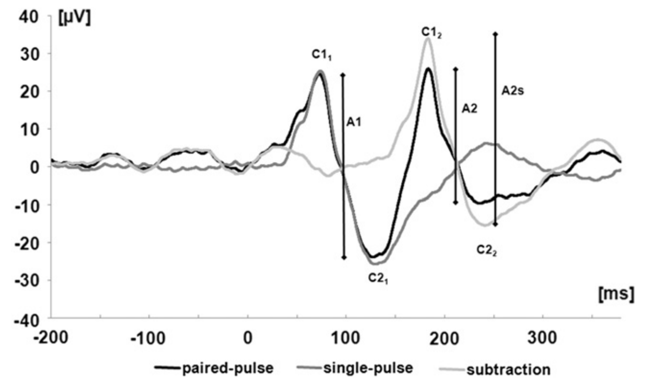

Figure 1. Visually evoked potentials over cortical Oz of one subject after single (dark grey trace) and paired-pulse stimulation with SOA of 107 ms (black trace). The denotation C was used to characterize the positive and negative components of the first and second response. The light grey trace results by subtracting the single-pulse trace from the paired-pulse trace. The analyzed amplitudes of the first response ($A1 = C2_1 - C1_1$) and second response ($A2 = C2_2 - C1_2$) after paired-pulse stimulation are marked by vertical bars; amplitudes of the second response after subtracting the response to a single pulse are denoted as A2s.

Experiment schedule

The participating subjects were randomly assigned to two groups. In one group TMS-induced phosphene thresholds was tested first and then (in a second session) paired-pulse stimulation was performed. In the other group the order of the sessions was inverted.

Statistical analysis

We used unpaired, two-tailed *t*-tests to analyse differences of averaged SOAs and single VEP amplitudes between subjects with and without phosphene perception. Paired, two-tailed *t*-test was used to test for differences between amplitude-ratios of both SOAs and amplitudes of first and second response of VEP. Significance was assumed at the $P = 0.05$ level. Before using parametric tests, normal distribution was confirmed using the Kolmogorov–Smirnov test, and homogeneity of variances was confirmed by *F*-test. In order to show correlation between phosphene thresholds and averaged amplitude-ratios, as well as between both amplitude-ratios we performed a linear bivariate correlation analysis (two-tailed Pearson's correlation). All calculations were performed using SPSS 17.0 software package (SPSS software, Munich, Germany).

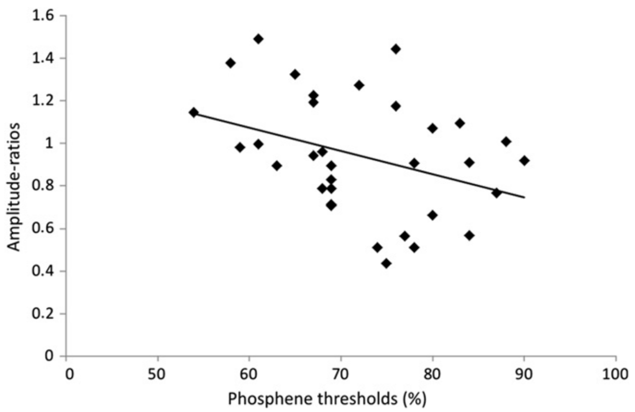

Figure 2. Linear bivariate correlation analysis of phosphene thresholds in % of maximal stimulator output (*x*-axis) and amplitude-ratios after paired-pulse VEP stimulation (*y*-axis) with linear regression.

Results

Overall, 17 out of 50 participating subjects (34%) were unable to perceive phosphenes even at maximum stimulator output and hence no phosphene thresholds could be determined. The remaining 33 subjects reported accurate and consistent phosphenes.

In this remaining group, the mean tested phosphene threshold was 72.3 $\pm$ 9.2% of the maximal stimulator output with a range from 54 to 90%.

Paired-pulse ratios at SOAs of 107 ms and of 133 ms were statistically not different with $P = 0.342$, but were significantly correlated ($r = 0.5$, $P < 0.005$). The pooled ratios (averaged from SOAs 107 ms and 133 ms) ranged from 0.44 to 1.49 (mean 0.94 $\pm$ 0.28). We found a statistically suppressive effect with a reduced second mean response in VEP compared to the first one after applying paired stimuli ($P < 0.001$).

Correlation analysis revealed a significant negative correlation between phosphene thresholds and averaged amplitude-ratios ($r = -0.36$, $P = 0.039$) (see Fig. 2).

The averaged SOAs in subjects without phosphene perception were 0.94 $\pm$ 0.29 (range 0.45–1.61). The averaged SOAs statistically did not differ between subjects with and without phosphene perception with $P = 0.95$.

Using *t*-test we found no statistical difference of single VEP amplitude (C1/C2-response) between subjects with (31.3 $\pm$ 13.8 μV) and without phosphene perception (35.5 $\pm$ 18.0 μV) with $P = 0.364$.

Discussion

This is the first study to investigate the relation between frequently used excitability markers of human visual cortex: TMS-induced phosphene thresholds and amplitude-ratios after VEP paired-pulse stimulation. We found a slightly, but significant negative correlation between both excitability markers. The higher the phosphene thresholds, the smaller were paired pulse ratios of paired-pulse VEPs, which signal low levels of cortical excitability, and vice versa. Our results are in accordance with findings in patients suffering from migraine [13,25], where reduced phosphene thresholds have been reported [2,26,27] as well as reduced paired-pulse suppression indicative of enhanced excitability [13]. Although it is conceivable that both approaches target aspects of visual cortex excitability, and hence reflect a common characteristic of visual cortex, both techniques may be mediated through different underlying mechanisms.

TMS-induced phosphenes evoke varying interindividual sensations of visual field disturbance ranging from brief and definable light sensations to moving or colour changing clouds in the visual field, the origin and underlying mechanisms, however, are still not clarified. There is evidence that phosphenes are generated in the primary visual cortex V1 cortex [10,11,28,29] and in extrastriate visual-cortical areas V2/3 [10,29,30]. Furthermore, an involvement of subcortical areas is described as a possible target of single TMS pulses to induce phosphenes perception [31,32]. In addition, other factors influence phosphenes perception like coil orientation, pulse configuration (mono- or biphasic) and pulse duration [10].

In our study presented here 34% of all subjects were not able to perceive phosphenes, even at maximum stimulator output. While this percentage is in accordance with other studies, there is still no conclusive explanation why some subjects fail to perceive phosphenes [10,11]. A possible limitation of phosphene perception has been attributed to interindividual variations in the morphology and topography of the stimulated areas caused by the depth of the induced electric field, or individual variability in the folding of the occipital gyri [33]. Sparing et al. suggested that interindividual functional differences of visual neuronal networks might also play a role for the induction of phosphenes [11].

Meister et al. found differences in fMRI activations of early visual cortex and VEP amplitudes in response to a standard checkerboard pattern between subjects who perceived phosphenes compared to those who did not [34]. In studies using TMS-induced phosphenes a higher prevalence of phosphene perception and reduced phosphene threshold in patients suffering from migraine compared to a healthy control group were reported (overview see [25]). Therefore, the lack of phosphene perception has been interpreted as an indication of low excitability level in visual cortex. However, we did not find a statistically difference of excitability assessed by paired-pulse VEP stimulation depending on phosphene perception. Hence, according to our findings, healthy subjects with and without phosphene perception seem might not differ in the level of visual cortex excitability.

In contrast to the detection and evaluation of TMS-induced phosphenes, which depends to some extent on subjective factors and the compliance of the subjects, amplitude-ratios of paired-pulse VEPs can be measured in every healthy subject.

Based on studies in both animals and humans, there is agreement that VEPs reflect population synaptic currents, while topographic studies using fMRI and electrical mapping in adult humans provide strong support that the first major component of the VEP elicited by a pattern onset stimulus (C1) arises primarily from parvocellular regions of primary visual cortex (V1). The C2 and C3 component of the VEP seem to have an extrastriate origin [35–37]. Despite substantial experimental and theoretical work across all sensory modalities, the mechanisms mediating PPS are not fully understood. There is agreement that presynaptic mechanisms play a crucial role [38,39]. In rat auditory cortex, for SOAs longer 100 ms, synaptic depression is assumed to play a crucial role [40]. In the visual cortex, suppression is also more consistent with thalamocortical synaptic depression than with inhibition [41,42]. In addition, there is evidence for an involvement of $GABA_B$ receptors [43]. Besides the contribution of GABAergic mechanisms, there is also evidence for the involvement of glutamatergic transmission in PPS [44,45]. Because of differences in PPS between cortical and thalamic cells, it has been argued that inheritance of thalamic response properties is unlikely to account for long-lasting forward suppression [40].

The major difference of both methods is, that applying TMS to induce phosphenes evokes a complex pattern of excitation and inhibition through the artificial transsynaptic stimulation of striate and extrastriate areas [46]. Contrary, paired pulse VEPs reflect the activation of neurons in primary visual cortex following presentation of physiological stimuli that is transmitted via the afferent sensory pathway. Taken together, both methods are useful to explore visual cortex excitability, and to characterize plastic changes in visual cortex. Both approaches may therefore reflect common characteristics of visual cortex excitability, but each approach most likely targets different mechanism. A combination of both approaches may provide a better understanding of excitability changes in visual system.

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