1. Introduction

Phosphenes are elementary light perceptions which can be elicited by transcranial magnetic stimulation (TMS) over the occipital cortex (Meyer et al., 1991; Marg and Rudiak, 1994). Phosphene threshold, which is the minimum magnetic stimulation intensity capable of eliciting phosphenes has been used as a measure of the excitability of the visual cortex (Afra et al., 1998; Aurora et al., 1998). In addition, phosphene perception has been utilized to assess short-term plasticity of the visual cortex and its underlying mechanisms (Boroojerdi et al., 2000a, b, 2001).

Single pulse focal TMS does not elicit phosphenes in all normal subjects. The percentage of investigated subjects perceiving phosphenes varies across studies. Our results, along with those of Meyer et al. (1991) indicate that about two-thirds of the subjects tested with TMS report phosphenes. The mechanism underlying the absence of phosphene perception in the remaining third is still unknown.

Furthermore, the neural correlates underlying phosphene perception are not well understood. Studies investigating the cortical site of phosphene perception using different TMS coil positions are yet to determine the exact site in the visual system (Kammer, 1999; Kammer et al., 2001). Although it is widely agreed that phosphenes are evoked within the central visual system, the exact mechanism of phosphene perception could not be characterised, mainly due to methodological limitations.

The present study adopted a new approach to further characterise the neural correlates of phosphene perception. Whereas, previous studies investigated only those subjects who reported phosphene perception, we sought to investigate differences in visual cortex excitability between subjects with, and without, phosphene perception. We addressed this issue by combining the TMS data with functional magnetic resonance imaging (fMRI) measurements of the activated visual network in response to a standard checkerboard stimulus. Previous studies locating the primary motor area of the hand using TMS mapping and fMRI activation found a high coherence between the two methods (Krings et al., 1997, 2001a, b; Boroojerdi et al., 1999), and TMS and fMRI can be seen as complimentary approaches in the evaluation of cortical
function. We utilized the comparison of visual fMRI activation between subjects with, and without, phosphene perception to determine the possible cortical areas, where TMS phosphenes are elicited. As an additional method to investigate the properties of the cortical visual system in the two groups, we used Visual Evoked Potentials (VEP) to measure the excitability of the visual system (Bonmassar et al., 1999; Kashikura et al., 2001). Recent studies have demonstrated that the dominant component of the VEP, the N1 component, is generated in the primary visual cortex (Slotnick et al., 1999; Di Russo et al., 2002), whereas the origin of the early P1 component lies within dorsal extrastriate cortex. Thus, the N1-P1 amplitude reflects the excitability of both striate and early extrastriate areas. However, Slotnick et al. investigating retinotopic maps of the primary visual cortex with VEP, showed that N1 is the major component of the VEP amplitude.

2. Methods

2.1. Subjects

We investigated 27 healthy normal volunteers (18 men, nine women, age 19–36 years). All subjects gave written informed consent and the protocol was approved by the local ethics Review Board.

2.2. Transcranial magnetic stimulation

During the determination of phosphene perception, subjects were blindfolded in a dark room. TMS was applied over a grid of nine points centered over Oz, according to the International 10-20 EEG electrode system, each point 2 cm apart. Magnetic stimulation was applied using a Magstim 200 magnetic stimulator (Magstim Co, Whitland, UK), equipped with a figure-of-8 coil (outer diameter of each wing 9.8 cm). Phosphene perception was tested with single pulse TMS with up to 90% of maximum stimulator output over all grid points. The subjects were asked to indicate the perception of phosphenes and the stimulation site where a given TMS intensity evoked the brightest phosphene. This point was determined as the optimal point for eliciting phosphenes. The phosphene threshold was then determined as the minimum intensity over the optimal point capable of eliciting phosphenes in three out of five trials. As a control, subjects who reported phosphenes also received TMS applied additionally over P3 and P4, and sham stimulation was performed over the optimal point (coil tilted away from the head to reproduce the stimulator sound and the cutaneous perception without brain stimulation). Subjects reporting phosphenes in one of the control conditions were excluded from further studies.

2.3. Functional magnetic resonance Imaging

Twenty-two subjects participated in the fMRI experiment, 11 of whom reported phosphenes while 11 lacked phosphene perception.

The cerebral activation was studied with functional magnetic resonance imaging employing blood oxygen level-dependent contrast on a 1.5 T Philips Gyroscan scanner (Philips Co., Best, The Netherlands) with a standard headcoil. An epoch design was used with eight experimental epochs (checkerboard) and eight baseline epochs (black screen with a central fixation crosshair). The fMRI sessions comprised four Dummy scans followed by 96 whole-brain scans (four scans for the checkerboard and eight scans for the baseline epochs) using single-shot gradient-refocused echo-planar imaging (EPI) (TR = 3.2 s, TE = 50 ms, flip angle = 90°, 24 slices). During the experimental condition, a checkerboard alternating with a frequency of 8 Hz was presented via MRI compatible high-resolution 3D glasses. The frequency of 8 Hz for the fMRI experiment was chosen to optimize the fMRI BOLD signal. In a previous study (Hoge et al., 1999) comparing different frequencies for a checkerboard, 8 Hz turned out to elicit a larger BOLD signal than 1 Hz. A checkerboard of 10 horizontal and eight vertical fields was chosen comprising a 30° field of view; it was presented at a resolution of 1,024 × 768 pixels. The subjects were asked to look carefully at the presented checkerboard.
2.4. **Visual evoked potentials**

Visual evoked potentials were measured in 16 subjects (eight reporting phosphenes, eight without phosphenes perception). The VEP were evoked by an alternating checkerboard pattern (frequency 1.3 Hz); the cortical response was measured using an electrode placed 4 cm above inion in the midline and referenced to an electrode over Fz (according to the International 10–20-EEG electrode system) using a Nicolet Electrodiagnostic System (Nicolet Co, USA). Each VEP was averaged across 120 single runs for both eyes separately. For further analysis the VEP amplitude (N1-P1 amplitude) was determined.

2.5. **Statistical analysis**

2.5.1. fMRI data

The fMRI data were analysed using Statistical Parametric Mapping software (SPM99, www.fil.ion.ac.uk/spm, London, UK). The dummy scans were discarded. The remaining scans were realigned and spatially normalised to a stereotactic space using an EPI-template (Montreal Neurological Institute (MNI), www.bic.mni.mcgill.ca/brainweb). The voxel size was $1.5 \times 1.5 \times 1.5$ mm. The normalised data were smoothed using a Gaussian kernel of $3 \times 3 \times 3$ mm in order to improve the signal-to-noise ratio. For the following parameter estimation an appropriate design matrix was specified using a box-car function as reference waveform. According to the general linear model, the voxel-by-voxel parameter estimation for the smoothed data was carried out. In order to test hypotheses about regionally specific effects, the resulting estimated beta-maps were compared by means of linear contrasts of each active and control condition. From this analysis resulted a map of $t$-statistic values (SPM(t)-map). To correct for the inference drawn by multi-subject fMRI data, a random effect model was applied (Friston et al., 1999), comparing the raw data of the subjects with a one-sample-$t$-test ($p = 0.001$). The resulting activations were corrected within boxes of $30 \times 30 \times 30$ mm around activated voxels found to be activated in previous studies (Bonmassar et al., 1999; Kashikura et al., 2001).

2.5.2. TMS and VEP data

The data of the two groups of subjects were analysed separately. To investigate those regions which were activated in subjects perceiving phosphenes but not in subjects lacking phosphenes, a masked activation map was created out of the SPM(t)-maps of the contrasts “checkerboard vs. baseline” of the two group activation maps resulting in a SPM(t) map “phosphenes perception [masked by] no phosphenes perception”.

3. **Results**

3.1. TMS and VEP data

Fifteen of the subjects (56%) tested reported phosphenes perception, 11 (41%) lacked any phosphenes perception, while one subject was excluded because she did not report phosphenes consistently. In the phosphenes perceiving subgroup, the average phosphenes threshold was 58.1% (3.36% (SEM)) of the maximum stimulator output. Only one subject reported Oz as optimal stimulation site, whereas in the other subjects the lateral stimulation sites were optimal (seven subjects right side vs. three subjects left side). The optimum point was located above Oz in three and below Oz in five subjects. None of the subjects showed optimal stimulus sites at points of the grid in the midline rostral and caudal to Oz. All subjects perceived phosphenes contralaterally, most describing them as small spots of light, while in some cases there were larger
phosphenes commonly described as stripes in the contralateral hemifield.

The average VEP amplitude was 5.03 ± 0.78 µV (SEM) for phosphene perceiving subjects and 9.09 ± 1.25 µV for the non-perceiving group. VEP amplitudes were significantly higher in the group lacking phosphene perception than in the group perceiving phosphenes ($t(7) = 3.17, p = 0.016$) (Fig. 1). The latencies of the N1 and P1 component did not differ significantly between the two groups (no phosphene group N1: 68.82 ± 1.69 ms (SEM), P1: 104.51 ± 1.93 ms; phosphene group N1: 70.33 ± 2.52 ms, P1: 101.74 ± 2.3 ms; $p > 0.1$).

3.2. MRI data

Analysis of the fMRI data revealed bilateral activations comprising the whole striate and extrastriate visual network in response to the checkerboard pattern for both subject groups (Fig. 2). Analysis of the cluster size and the peak activations in the group data showed that the network activated in the phosphene perceiving group was larger than in the non-perceiving group (total size of the clusters activated in the visual network was 1,870 voxels in the phosphene group and 1,026 voxels in the no phosphene group). In contrast, the peak fMRI activation, which was located in the striate cortex in both groups, was slightly higher in the group lacking phosphene perception compared to the perceiving subjects ($t$-value phosphene group: $t = 12.83$; no phosphene group: $t = 14.53$, $p > 0.1$).

The analysis using a masked activation map to determine the regions which were activated solely in the phosphene group but not in the no phosphene group revealed that bilateral extrastriate areas comprising Brodmann Area 18 and 19 (V2, V3) were activated in association with phosphene perception (Fig. 3).

There was no significant correlation between phosphene threshold and size of the whole activated cluster in fMRI ($r = 0.169$). Because it is not possible in SPM to determine the size of subclusters belonging to a certain Brodmann Area within a greater cluster,

![Fig. 1. Visual Evoked Potentials. Left, examples of right-eye VEP of a subject lacking phosphene perception (above) and a subject reporting phosphenes. Right, the average VEP amplitudes of the two groups. Error bars indicate standard errors.](image1)

![Fig. 2. fMRI group activations of subjects without (above) and with phosphene perception (bottom). Whereas subjects reporting phosphenes activated a larger visual network in response to checkerboard stimuli, the peak amplitude was slightly (not significantly) higher in subjects lacking phosphene perception.](image2)
we performed a correlation analysis between the size of the whole activated fMRI cluster including V1 and phosphene threshold.

4. Discussion

The present study revealed remarkable differences in visual network activation in response to a standard checkerboard pattern between subjects perceiving phosphenes and those lacking phosphene perception. The activated bilateral visual network in response to the standard visual stimulus was larger in subjects who perceived phosphenes, comprising a greater part of the extrastriate cortex. Both the peak activation measured with fMRI and VEP amplitudes, however, were higher in subjects who did not report phosphene perception. This suggests that the excitability of the primary visual cortex (and possibly early extrastriate areas) is higher in subjects who do not perceive phosphenes, whereas the excitability of higher extrastriate areas is higher in subjects who do report them. In our study, N1-P1 VEP amplitude was measured. It has been shown before, that generators in V1 and early extrastriate areas contribute to this amplitude (Di Russo et al., 2002). Therefore, a conclusive distinction between V1 and early extrastriate excitability is not possible using N1-P1 VEP amplitude.

Although a wide range of paradigms have been employed for phosphene induction, and it is generally accepted that phosphenes are generated in the central visual system and provide a measure of visual cortex excitability, there is no consistent theory concerning the anatomical site where TMS induced phosphenes are elicited. Meyer et al. (1991) performed a topographical analysis of phosphenes under different TMS coil positions, asking subjects to rate the brightness of the induced phosphenes. Comparing the position of the coil with the phosphene location within the visual field reported by the subjects, they concluded that the rostral part of the calcarine sulcus may be the region where TMS induced phosphenes are likely to be generated.

Marg and Rudiak (1994) used a different approach to the problem: in their study, two TMS coils of different diameters were used to evoke phosphenes. TMS intensity was adjusted to induce phosphenes of the same brightness with both coils. Comparison of the electrical field revealed a depth of stimulation of 4 cm, suggesting the optic radiation as locus of phosphene perception. This was supported by the finding that phosphenes were reported to the same extent in the periphery and the foveal part of the visual field.

Three recent studies (Cowey and Walsh, 2000; Fernandez et al., 2002; Gothe et al., 2002) have employed transcranial magnetic stimulation in blind subjects. In the first study, TMS was applied to sighted subjects, one retinally blind subject and one subject lacking V1 in one hemisphere due to trauma at young age. Whereas the retinally blind subject reported phosphenes, the latter subject did not report phosphene perception when stimulated over the damaged hemisphere. Cowey and Walsh claimed that an intact V1 is essential for phosphene perception. However, it remains unclear if an intact V1 is also sufficient for phosphene perception. The second and third studies have shown that phosphene perception may be present in peripherally blind subjects. In the study of Fernandez et al., 13 blind participants were studied, seven of whom were able to perceive phosphenes. Gothe et al. (2002) compared phosphene perception in subjects with or without residual vision and fully sighted individuals and found that phosphenes could be elicited in all sighted subjects and those with residual vision but only in 20% of blind subjects. The two latter studies included only

Fig. 3. Three slices showing fMRI activations, which were only present in subjects perceiving phosphenes but not in subjects lacking phosphene perception. This activation comprises part of the extrastriate cortex.
subjects with blindness due to pregeniculate lesions preserving the integrity of the occipital cortex without visual input.

The fact that phosphenes can be elicited in subjects suffering from blindness due to pregeniculate lesions show that phosphenes perception is independent of intact visual perception, however, the phosphenes threshold is usually higher than in healthy controls.

In accordance with the studies by Cowey and Walsh (2000), Kammer et al. (2000) noted that phosphenes can be induced from multiple target sites over the striate and extrastriate (V2/V3) cortex without qualitative differences, and concluded that either the target structure for TMS is the optic radiation or that the extrastriate cortex evokes activity in V1 which results in phosphenes perception.

Taken together, there are several hypotheses but no clear evidence concerning the cortical site of phosphene perception. In the present study a standard alternating checkerboard stimulus elicited a stronger BOLD response in V2/V3 in the phosphene group compared to the no phosphene group which favours an involvement of the extrastriate cortex in phosphene perception. If, as suggested by Cowey and Walsh (2000), V1 is also critical for phosphene perception, then phosphenes could be a product of signals originating in V1 (or the optic radiation) which are then transmitted to secondary visual areas.

Our finding that subjects lacking phosphene perception tend to have a higher excitability in V1 would be in accordance with the notion that only a certain level of excitability in V2/V3, where the incoming information from V1 is processed, can lead to perception of phosphenes. This is supported by the finding of Fernandez et al. (2002) that phosphenes differ remarkably in size, from a “pinpoint” to almost the whole visual field, and that phosphene size is not correlated with phosphene intensity: if there were a distinct cortical site within V1 generating phosphenes, one would expect a greater uniformity of phosphene perception.

In conclusion, subjects reporting phosphenes activate a larger extrastriate cortex network when exposed to a standard checkerboard stimulus compared to subjects lacking phosphene perception. Thus, the excitability of the extrastriate cortex seems to be related to phosphene perception, whereas the level of striate cortex excitability does not play a critical role for phosphene perception. This has implications on studies using phosphene threshold to measure the excitability of the visual cortex: studies which found short-term plasticity within the visual system due to light deprivation (Boroojerdi et al., 2000a) or changes of phosphene threshold in migraine patients (Aurora et al., 1998) measure changes likely to occur also in the extrastriate cortex. However, properties of phosphene perception need to be further characterised. An interesting approach would be to investigate phosphenes using separate TMS stimuli to V1 and V2/V3 at different time intervals.

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