Subjective Characteristics of TMS-Induced Phosphenes Originating in Human V1 and V2

Niina Salminen-Vaparanta1,2, Simo Vanni3,4, Valdas Noreika1,2, Vladas Valiulis5, Levente Móró1,2 and Antti Revonsuo1,2,6

1Centre for Cognitive Neuroscience, 2Department of Psychology, University of Turku, Finland, 3Advanced Magnetic Imaging Centre, 4Brain Research Unit, O.V. Lounasmaa Laboratory, Aalto University School of Science, Finland, 5Department of Neurobiology and Biophysics, Vilnius University, Lithuania and 6School of Humanities and Informatics, University of Skövde, Sweden

Address correspondence to Niina Salminen-Vaparanta, Centre for Cognitive Neuroscience, University of Turku, FIN-20014 Turku, Finland. Email: niisalm@utu.fi

One way to study the neural correlates of visual consciousness is to localize the cortical areas whose stimulation generates subjective visual sensations, called phosphenes. While there is support for the view that the stimulation of several different visual areas in the occipital lobe may produce phosphenes, it is not clear what the contribution of each area is. Here, we studied the roles of the primary visual cortex (V1) and the adjacent area V2 in eliciting phosphenes by using functional magnetic resonance imaging-guided transcranial magnetic stimulation (TMS) combined with spherical modeling of the TMS-induced electric field. Reports of the subjective visual features of phosphenes were systematically collected and analyzed. We found that selective stimulation of V1 and V2 are equally capable of generating phosphenes, as demonstrated by comparable phosphenes thresholds and similar characteristics of phosphenes shape, color, and texture. However, the phosphenes induced by V1 stimulation were systematically perceived as brighter than the phosphenes induced by the stimulation of V2. Thus, these results suggest that V1 and V2 have a similar capability to produce conscious percepts. Nevertheless, V1 and V2 contribute differently to brightness: neural activation originating in V1 generates a more intense sensation of brightness than similar activation originating in V2.

Keywords: brightness, functional magnetic resonance imaging, primary visual cortex, transcranial magnetic stimulation, V2

Introduction

Transcranial magnetic stimulation (TMS) and stimulation via intracortical electrodes of early visual cortex induces phosphenes, short-lived subjective visual sensations (Brindley and Lewin 1968; Meyer et al. 1991). This basic form of visual experience emerges with varying stimulation thresholds in different individuals (Kammer et al. 2005). There is also large interindividual variation in the characteristics of phosphenes. Typically, phosphenes are described as gray or white, but they can also contain unsaturated colors, and they can have various shapes, such as arc, blob, oval, sector, line, and so forth. (Marg and Rudjak 1994). The location of phosphenes corresponds roughly with the retinotopical organization of the primary visual cortex (V1, Dobelle and Mladjovsky 1974), and the size of phosphenes increases with increasing TMS intensity (Kammer et al. 2005).

Phosphenes can be generated by the stimulation of several different visual areas which are at the low level of anatomical cortical hierarchy. For instance, electrical stimulation via microelectrodes implanted in and close to the calcarine fissure generates phosphenes (Brindley and Lewin 1968; Dobelle and Mladjovsky 1974; Schmidt et al. 1996). While in an early study Dobelle and Mladjovsky (1974) reported that Brodmann area 17 (V1) is the most effective locus to produce phosphenes, later studies have shown that phosphenes can be also induced via intracranial electrodes placed in various locations in the occipital and temporal lobes of epilepsy patients (Lee et al. 2000; Murphey et al. 2009). In addition, Kammer et al. (2001) demonstrated that phosphenes can be elicited when the center of the TMS coil is placed 5 cm laterally from the mesial occipital cortex in neurologically healthy participants, which would target extrastriate areas (Hasnain et al. 1998). However, the TMS of an extrastriate area did not induce phosphenes in a patient lacking a part of the ipsilateral V1, which was suggested to imply that an intact V1 is required for TMS-induced phosphenes (Cowey and Walsh 2000).

It is uncertain whether phosphenes produced by the stimulation of V1 are similar to those induced by the adjacent extrastriate areas. Kammer et al. (2005) aimed to compare the TMS-induced phosphenes by identifying the early visual areas with functional magnetic resonance imaging (fMRI) and placing the TMS coil above one of the target areas (V1, V2d, or V3d). Participants’ task was to draw on the screen the contour of phosphenes they saw. However, Kammer et al. (2005) found no differences in the subjective characteristics of phosphenes between the areas. As the authors themselves concluded, it is possible that the method was not sufficiently accurate to determine which area was stimulated because no modeling of the TMS-induced electric field (E-field) was applied. Murphey et al. (2009) identified the early visual areas with fMRI to guide the intracranial electrical stimulation and asked participants to describe size, location, color, and complexity of phosphenes, but in line with the results by Kammer et al. (2005), they did not find systematic differences in any characteristics of phosphenes between the early visual areas. In contrast, Lee et al. (2000) stimulated patients with cortical electrodes, and reported that the shapes of phosphenes were more blob-like around the calcarine sulcus, and became more complex further away from the calcarine fissure. However, they identified the functional areas by relying on purely anatomical landmarks, so it was not clear which functional visual areas produced the more complex phosphenes. The differences in the subjective characteristics of phosphenes between different visual areas have never been directly tested in neurologically healthy humans with sufficiently accurate stimulation methods and by using systematic analyses of subjective ratings.

Recently, a novel experimental procedure was introduced which allows targeting TMS to a specific early visual area in humans, in particular V1 or the adjacent extrastriate area V2, which enables causal probing of differences in their functional roles (Salminen-Vaparanta et al. 2012). In this experimental procedure, multifocal identification of early visual areas (Vanni
et al. 2005) is combined with the spherical modeling of the TMS-induced E-field. Evidently, compared with the stimulation via intracranial electrodes, the spatial resolution of neuronavigated and modeled TMS is still poor (~5–7 mm at its best). Nevertheless, compared with ordinary TMS or to direct electrical stimulation, the new TMS procedure has the advantage that neurologically healthy human participants can be tested according to the individually determined functional maps of the early visual areas.

The aim of the present study was to directly test whether the phosphene reported by V1 stimulation as compared with those produced by V2 stimulation systematically differ from each other in their subjective visual features. We reasoned that this experimental set up might reveal valuable information concerning the causal roles of V1 and V2 in evoking phenomenal contents of visual awareness and thereby also contribute to the search for the neural correlates of visual consciousness (Koch 2004). This study design would also go beyond the Kammer et al. (2005) study because we determined the target areas for stimulation by using detailed retinotopic maps of V1 and V2, targeted the TMS pulses with the help of E-field modeling and, most importantly, systematically collected subjective reports of the induced experiences with the Phosphenes Questionnaire which was developed for this study. By contrast, Kammer et al. (2005) defined only the borders between the early visual areas for stimulation, did not use the E-field modeling in targeting the TMS, and based the subjective phosphene reports on drawings and free recall.

Materials and Methods

Participants

Five individuals were selected from a larger database of 10 fMRI-tested participants on the basis that their V1 and V2 were located optimally close to the head surface for selective TMS. They were tested to ensure they are giving reliable phosphene reports so that their reports should systematically co-vary with the TMS while participants were blind to the stimulation condition: they should not report seeing phosphenes when TMS was below the intensity required to induce phosphenes, the number of phosphene reports should increase with increasing TMS intensity, and when the coil location was kept the same they had to report phosphene threshold. Thus, 4 neurologically healthy students of the University of Turku (age 22–28 years, 2 males) completed the TMS experiment. All participants had normal or corrected-to-normal vision. Each participant gave their written informed consent and the Ethical Committee of The Hospital District of Southwest Finland approved the protocol. The participants were naive to the purposes of the experiment and they were paid for their participation.

Retinotopic Mapping of Areas V1 and V2

The fMRI paradigm of the present study has been reported earlier (Salminen-Vaparanta et al. 2012). A 3T MRI scanner (Signa™ HDxt, General Electric, Inc., WI, USA) was used for data collection, with phased-array 8-channel head coil. The major parameters for functional imaging were repetition time 1800 msec, echo time 30 ms, matrix 64 x 64, flip angle 60°, FOV 160 x 160, and slice thickness 2.5 mm. Anatomical images were also gathered for each participant (matrix 256 x 256, FOV 250 x 250, and slice thickness 0.9 mm).

Multifocal fMRI design (Vanni et al. 2005) was used in determining the retinotopical organization of V1 and V2 for each participant. The visual stimuli were projected with data projector ( Christie X3™, Christie Digital Systems Ltd, Mönchengladbach, Germany) by using Presentation™ software (Neurobehavioral Systems, Inc., Albany, CA, USA). The multifocal fMRI design comprised 24 subareas (3 rings extending at 1–3.2°, 3.2–6.7°, and 6.7–12° eccentricities, and 8 wedges, Fig. 1A) which were mapped for each participant. Each run was divided into 32 blocks, where approximately half of visual field regions were active simultaneously, with checkerboard reversing contrast at 8.3 Hz (dark 4 cd/m², light 40 cd/m²), or inactive showing uniform gray (22 cd/m²). Each visual field region was actively stimulated in half of the blocks, and the timing of the on-blocks followed a quadratic residue sequence, which provides linear independent timing for all visual field regions. A session included 4 runs, each with 132 volumes. Compared with the original design with 60 regions (Vanni et al. 2005), the reduced number of regions and presenting stimuli in 2 different sets of regions in 2 consecutive time intervals reduce suppressive neural interactions between the subareas (Pihlaja et al. 2008). By using this method, the 24 regions can be reliably mapped in the V1, V2, and V3 with the multifocal design (Henriksson et al. 2012; Salminen-Vaparanta et al. 2012).

We preprocessed the fMRI data with the SPM2 toolbox (Wellcome Department of Imaging Neuroscience, London, UK). The general linear model had one regressor for each of the 24 regions, plus a separate constant regressor for each of the 4 runs. A high-pass filter at 128 s cutoff attenuated low-frequency confounds, and an AR(1) model captured noise autocorrelation. The SPM t-maps were superimposed on an anatomical 3D image to define the borders between the early visual areas V1, V2, and V3 and to map the centers of each subarea in V1 and V2 (Fig. 1). SPM and Navigated Brain Stimulation (NBS) system (eXimia 2.1.1; Nexstim Ltd, Helsinki, Finland) function in the same coordinate system. All coordinates of the retinotopically mapped V1 and V2 subarea centers that were visually determined with SPM were thus entered to the eXimia NBS software for TMS targeting and E-field modeling (Fig. 2; see also Salminen-Vaparanta et al. 2012).

TMS and E-Field Calculation

TMS pulses were generated with the eXimia™ TMS magnetic stimulator (Nexstim Ltd) via figure-of-8 Nexstim monopulse coil (outer winding diameter =70 mm). Participant’s head was supported by a chin rest and the coil was clamped to a holder to keep it tight against the participant’s head. In the first experiment, the maximum frequency of TMS was 0.29 Hz, and in the second experiment, it was 0.26 Hz. To localize the TMS coil in relation to the brain, MRI-guided NBS system (eXimia 2.1.1; Nexstim Ltd.) was applied, which provided also the calculation of the intracranial E-fields of TMS pulses. The estimated spatial resolution of the NBS system is 5.7 mm when all sources of errors are considered (Ruohonen and Karhu 2010). Direction of the TMS-induced E-field was set according to anatomical maps, so that the strongest E-field would be directed perpendicular to the gyrus of target region. There is some evidence that the phosphene thresholds (the stimulation intensity required to evoke phosphenes) are lowest if the E-field is directed perpendicular to the sulcal bank (Fox et al. 2004; Kammer et al. 2007). TMS-induced E-field was modeled with spherical conductor model (Servais 1987; Heller and van Hulsteyn 1992) during each trial, to ensure that the E-field was higher in V2 than in V1 during the targeted stimulation of V2, and vice versa. In detail, the aim was that the E-field strength in the nontargeted V1 or V2 would be under the phosphene threshold intensity of that area. Thus, the E-field strength was above the phosphene threshold intensity only at the target area (Fig. 3). Given that during the phosphene threshold determination in Experiment 1 there were only a few trials with the intensity at the phosphene threshold level, the exact E-field strength value at the phosphene threshold intensity was calculated from the TMS-induced E-field strength during Experiment 2. This enabled to obtain more TMS trials for plotting the averaged E-field strength at the phosphene threshold.

Selecting the Target Sites in V1 and V2 for TMS

To increase the probability of hitting a target subarea in V1 or V2, while minimizing the E-field strength in the retinotopically homologous V2 or V1 subarea, the optimal position for the coil center (i.e., the optimal subarea of all 24 subareas) was selected based on the following criteria: (1) in V1 and V2, homologous subareas should be located
at maximum possible distance from each other; (2) they should lay at an equal distance from the scalp; (3) they should be as close to the head surface as possible; (4) they should lie on the radially oriented gyral surface and not in the sulci. For all participants, the selected subarea of V1 was in the right hemisphere, representing the lower left visual field region, 1–3.2° from the fixation (region 6, Fig. 1A). The same applied to V2 subareas for 3 participants, but for one participant the targeted V2 subarea was located in the left hemisphere, contralateral to her V1 stimulation.

**Experiment 1: Phosphene Thresholds**

In Experiment 1, phosphene thresholds for V1 and V2 stimulation were calculated for each participant. Phosphene thresholds were
defined as the stimulator output intensity which induces phosphenes in 50% of TMS pulse trials (Kammer et al. 2001; Deblieck et al. 2008). Participants were sitting in a dentist’s chair in a dimly lit room, in front of a widescreen 24” LCD monitor (Dell, Round Rock, TX, USA) screen at a 37-cm distance. Participants fixated on a gray cross (mean luminance 0.4 cd/m²) in the center of black background (mean luminance 0.3 cd/m²). They were instructed to give auditory feedback with a metal rod of a chin rest if they had perceived a phosphen. Each yes/no report was entered manually to a worksheet by the experimenter. First, stimulation intensity was set to 20% of the maximal output of the stimulator and it was increased after each pulse at a rate of 2% units until the first phosphene was reported. This stimulation intensity was marked as the lowest point for the following stimulation procedure, which involved applying TMS pulses with 10 different intensities, rising at the step of 2% units from the lowest point. Pulses were delivered in a randomized sequence 10 times at each stimulator output intensity, rendering 100 pulses for each area. For 2 participants during stimulation of V1 and for one participant during stimulation of V2, the phosphene threshold curve was more gradual, never reaching 50% of phosphene reports. For these participants, the aforementioned procedure was repeated by starting from the highest tested intensity in order to reveal an intensity with 50% of phosphene reports. Thus, for these 3 participants either V1 or V2 received 200 pulses.

Phosphene reports for each stimulation intensity were summed. The data were then fitted to a sigmoidal (Boltzmann) function using Origin software (OriginLab, Northampton, MA, USA), and the phosphene threshold value was taken from the sigmoidal function (Kammer et al. 2001; Fig. 4).

Experiment 2: Assessment of Phosphene Quality
In Experiment 2, 100 phosphenes were elicited for each participant by stimulating V1 and another 100 phosphenes were induced by stimulating V2, both at TMS intensity 110% relative to the individual phosphene threshold. The participants did not know the stimulated cortical area. Regarding the apparatus, viewing distance and behavioral...
of 10 delivered pulses. The threshold was defined as the half-maximal value (5/10) of the fitted function (dotted line). Measurements comprised 20 different TMS pulse intensities in steps of 2% stimulator output intervals, which were set in a pseudorandomized order.

Responses, Experiment 2 was similar to that in Experiment 1. In addition, after each positive phosphene report, participants drew a picture of the perceived phosphene on the screen with a tablet pen, and after each drawing, the Phosphene Questionnaire form (letter mean luminance 2.7 cd/m²) was presented on the screen and the participant had to fill it in by using the same tablet pen (see Supplementary Fig. S1). Experimental setup included 5 blocks of 20 positive phosphene reports for each area, resulting in 200 positive phosphene reports for the 2 areas. The order of V1 and V2 stimulation was pseudorandomized. The data of Experiments 1 and 2 were collected in 4 or 5 TMS sessions each of which lasted between 1.5 and 3 h.

A total of 800 questionnaire forms and 800 phosphene drawings were collected from the participants, 400 from stimulation of V1 and another 400 from stimulation of V2. Data processing of the images was automated and the required programming was implemented in PERL language scripts in a Debian Linux environment using GraphicsMagick image processing utilities. For converting the hand-marked Phosphene Questionnaire forms into numerical data, an optical symbol recognition system was developed. First, the questionnaire files were converted into Portable BitMap (PBM) format, so that the question texts of the form were merged into the background, leaving only the answer marks visible. Then for each question, all of its answer options were automatically checked for any marks in the corresponding answer boxes.

Based on the Phosphene Questionnaire, we formulated 27 research questions regarding the quality of phosphenes. In particular, we compared between V1 and V2 stimulation 1) the number of different shapes in single stimulation, 2) the number of inside stuffed phosphenes, 3) the number of inside empty phosphenes, 4) the number of phosphenes locating partly outside the screen, 5) the number of phosphenes with just one color [red, green, blue, yellow, purple, brown, orange] or shade [gray, white or black] in single phosphene perception, 6) the number of achromatic phosphenes, 7) the ratings for the brightness of phosphenes, 8) the number of phosphenes in single stimulation, 9) the number of phosphenes with shape of dot/triangle/square/line/arc/angle/object, (10) the number of phosphenes with color of red/green/yellow/purple/white/brown/gray/black/orange. In addition, the total amount of unique hues in phosphenes was compared with the total amount of other hues in phosphenes. After Bonferroni correction, the significance level was adjusted to $P < 0.0019$ and only $P$ values lower than this were taken as statistically significant.

Mann–Whitney test was used individually for each participant to analyze the quantity of phosphenes in a single stimulation, the number of phosphenes with just one color or shade in a single stimulation, the number of different basic shapes in a single stimulation, the number of achromatic phosphenes, the total amount of unique hues versus other hues in a single stimulation and the brightness of phosphenes. Data of categorical phosphene features, such as exact colors, shapes, area (empty/filled inside), and location of phosphene were analyzed using $\chi^2$ test, which was again carried out individually for each participant. Some of the values, occurring in small numbers, were analyzed using Fisher’s exact test. If all participants showed similar difference for a specific feature of phosphenes between the phosphenes evoked from V1 or V2, we ran group analyses with paired-samples $t$-test despite the fact that the sample size was small, which would reduce the statistical power.

Participants reported their percepts also in phosphene drawings, revealing the precise location and extent of each phosphene. First, the subject’s actual visual field size was calculated both in degrees and in screen pixels. The drawings were checked against a visual mask consisting of an inner disc with a diameter corresponding to a 1° foveal area of the visual field, and 3 concentric rings at 4°, 8°, and 12° of the visual field. These 3 rings were divided into 845° sectors, from which 4 were divided further into 22.5° subsectors by the horizontal and vertical axes. Thus, the masking procedure defined a total of 24 sectors and 12 subsectors, as well as 12 corresponding areas outside the largest ring (see Supplementary Figs S2–S9). In an automated process, each phosphene drawing was checked against each sector, and the number of pixels found inside that sector was written into a text data file in CSV format. The generated CSV data files were converted into Microsoft Excel sheet (XLS) file format.

Results

The phosphene thresholds between V1 and V2 did not show any systematic difference (Fig. 3). For 2 participants, the phosphene threshold was higher in V1, whereas for the other 2 participants, it was higher in V2. The most consistent statistically significant difference between phosphenes elicited by V1 and V2 stimulation that unfolded separately for all tested participants was in the brightness of phosphenes: phosphenes evoked by V1 stimulation were significantly brighter than the phosphenes induced by V2 stimulation ($P < 0.000001$, Fig. 5). In addition, we also carried out a group analysis, which confirmed that the phosphenes were brighter when elicited by TMS of V1 than the phosphenes induced by TMS of V2 ($t_{(3)} = 8.230, P = 0.0038$).

In additional analyses, we compared the subjective ratings of the brightness of the phosphenes between and within the sessions to evaluate the reliability of the brightness ratings. Three participants participated in 2 sessions and 1 participant in 3 sessions. To compare brightness ratings between the first and the second sessions for participants P1, P2, and P3, Mann–Whitney $U$-tests were carried out, separately for V1 and V2. For participant P4, Kruskal–Wallis tests were carried out with 3 sessions as independent samples. In a case of significance, Mann–Whitney $U$-tests were used for pairwise comparison of 2 sessions. Given that 8 main tests were carried out for 4 participants (V1 and V2 separately), $P$ value was Bonferroni corrected to $0.05/8 = 0.00625$. Three comparisons out of 8 yielded significant differences between the sessions. For P1 and P3, the V2 stimulation gave different results in the first and second sessions in the brightness ratings ($P < 0.001$), and for P4, there was a main effect of session ($P < 0.001$), which was due to the different results in the third and preceding sessions in V1 stimulation ($P < 0.001$).
Spearman correlation tests were carried out to explore possible correlation between phosphene brightness ratings and trial numbers within each session. Positive correlation would indicate that brightness ratings increased during a session, while negative correlation would show that brightness ratings decreased during a session. Eighteen separate correlation tests for each participant, each session, and each brain area (V1 and V2) were carried out, and \( P \) value was Bonferroni corrected to 0.05/18 = 0.0028. Notably, P4 had 3 sessions, whereas the remaining 3 participants had 2 sessions each. Of 18 tests, only 1 yielded significant change of phosphene ratings: for participant P4, the brightness of V2 phosphenes increased during session 3 (\( \rho = 0.58, P < 0.0001 \)). Thus, phosphene brightness was very stable within a session (only 1 test was significant out of 18), whereas between session comparisons revealed more differences in phosphene brightness (3 tests significant out of 8). Given that the brightness of phosphenes was self-evaluated by a scale from 1 to 7, at the beginning of each session participants had to adjust their subjective judgments trying to cover the whole range of the scale. During this procedure, there is always some fluctuation in subjective perception and its evaluation (even if visual object stimuli are presented), which likely explains the differences between some of the sessions.

The other subjective characteristics of phosphenes induced from the stimulation of V1 and the stimulation of V2 were surprisingly similar and seemed to be more dependent on individuals rather than areas. Figure 6A shows the amount of colors in phosphenes. Two participants reported seeing almost only achromatic phosphenes (>99.5% of phosphenes were achromatic), whereas the other 2 participants saw also chromatic phosphenes (on average 56% of phosphenes were chromatic). One participant reported seeing more chromatic phosphenes than achromatic phosphenes evoked by TMS of V1 compared with phosphenes induced by TMS of V2 (\( P = 0.0010 \)). Another participant reported seeing more white phosphenes evoked by TMS of V1 compared with phosphenes induced by TMS of V2 (\( P < 0.001 \)). None of the chromatic phosphenes reported by these 2 participants contained the color orange and only a few times phosphenes contained brown or purple. By contrast, the colors red, green, blue, and yellow were frequently reported among the chromatic phosphenes. The difference between the frequencies of unique hues (i.e., red, green, blue, and yellow) and other hues in phosphenes was significant for participant P2 (\( P < 0.0001 \)) and was approaching significance for participant P1 (\( P = 0.024 \)).

All participants saw phosphenes with more than one color or shade in a single phosphene. Overall, 36% of all the 800 phosphenes contained more than one color or shade simultaneously and the rest of the phosphenes contained only one color or shade. There was a trend that phosphenes induced by TMS of V1 contained more shades or colors than the phosphenes induced by TMS of V2. The count of phosphenes with only one color or shade was 72/V1 and 82/V2 for P1; 53/V1 and 62/V2 for P2; 38/V1 and 40/V2 for P3; and 70/V1 and 93/V2 for P4. However, the difference between V1 and V2 was statistically significant only for participant P2 (\( P < 0.0001 \)).
The group analysis revealed $P$ value $0.087$ ($t_{(3)} = -2.513$) for the comparison of the V1 and V2 in number of phosphenes with only one shade or color. Furthermore, one participant showed statistically significant correlation between the brightness of phosphenes and the count of colors or shades in a single phosphene ($\rho = 0.46, P < 0.001$). The different shapes reported by the participants are presented in Figure 6B. The shape differences were more systematically related to the individual participants than to the stimulation of particular visual areas.

The results of the phosphene locations in the visual field showed that phosphenes were strongly dominated by the contralateral visual field relative to the stimulation site (see Supplementary Figs S2–S10). For 3 participants in more than 50% of trials, phosphenes appeared in the visual field areas that were 12–39° from the fixation. It is unlikely that the cortical representations of such areas would be stimulated directly with TMS due to their deep location in the visual cortex. For instance, there were specific visual field regions in which participants reported phosphenes in more than 50% of trials, but the E-field strength in the corresponding cortical representation was always below 50% of the phosphene threshold. In particular, for P1 E-field strength in region 21 was 44% of the phosphene threshold (72/166 V/m) during V1 stimulation; for P2 E-field strength in region 21 was 38% of the phosphene threshold (60/159 V/m) during V2 stimulation, for P3 E-field strength in region 22 was 46% of the phosphene threshold (62/139 V/m) during V1 stimulation, and for P4 E-field strength in region 21 was 40% of the phosphene threshold (70/175.5 V/m) during V1 stimulation. When we stimulated participants with these intensities at the beginning of the phosphene threshold determination, they did not report phosphenes.

**Discussion**

In this study, our aim was to compare the subjective characteristics of TMS-induced phosphenes between V1 and V2 stimulation. We found that based on subjective reports V1 and V2 phosphenes were very similar. The most prevailing and consistent difference in the characteristics of phosphenes was in their brightness, that is perceived luminance. The phosphenes induced by TMS of V1 were brighter than the phosphenes evoked by TMS of V2. This finding indicates that neural activation within V1 or neural activation via connections from V1 to other cortical areas generates more intense sensations of brightness than neural activation within V2 or via connections from V2 to the other areas. Given that the phosphene thresholds between V1 and V2 were similar, and therefore both early visual areas seem to be equally sensitive to TMS, the higher phosphene brightness after V1 stimulation cannot be attributed to higher stimulation intensity in this area.

Despite the fundamental role of brightness in visual awareness, its neural correlates are relatively poorly known in humans. While some human fMRI studies relate brightness perception with the activation of higher cortical areas than V1 or V2 (Cornelissen et al. 2006), the results from single-cell recordings in cats (Rossi et al. 1996) and monkeys (Kinoshita and Komatsu 2001) show that the activation of a subpopulation of V1 neurons is associated with a sensation of brightness. In addition, increase in the length of pulse sequence, pulse duration, or frequency of electrical stimulation of V1 produced brighter phosphenes in a patient who was blind due to glaucoma (Schmidt et al. 1996; for review, see Tehovnik and Slocum 2007).

Contrary to our results, Kammer et al. (2005) found no differences in the characteristics of phosphenes between V1 and V2. It is likely that the fMR image-guided TMS method that Kammer et al. (2005) used was simply too coarse to selectively stimulate V1. The isolated stimulation of V1 with TMS is complicated given that V1 is mostly folded into the calcarine fissure. To target and stimulate it accurately, the TMS-induced E-field needs to be controlled carefully (Thielscher et al. 2010; Salminen-Vaparanta et al. 2012). Respectively, by using fMR image-guided stimulation via intracranial electrodes, Murphey et al. (2009) did not report systematic differences in any characteristics of phosphenes between the visual areas. Unfortunately, they did not ask about the brightness of phosphenes from the participants. It is also noteworthy that in the studies by Kammer et al. (2005) and Murphey et al. (2009), the method to analyze subjective ratings of the different features of phosphenes (e.g., color, shapes) was qualitative rather than quantitative: groups based their results either on drawings and free recall (Kammer et al. 2005) or an interview and assessment of color and size of phosphenes (Murphey et al. 2009). It is possible that the scoring of some phosphenes' characteristics was not well controlled. Instead, in our study, we carried out systematic comparisons with the same Phosphen Questionnaire for each participant and analyzed the data statistically. In addition, a grading was applied to obtain quantitative data of the intensity of the brightness of phosphenes. Thus, our results suggest that systematic analyses of subjective ratings might reveal some information of the characteristics of phosphenes that could be missed with qualitative methods.

The phosphene thresholds between V1 and V2 were similar which is in line with earlier reports (Lee et al. 2000; Kammer et al. 2005; Murphey et al. 2009) implying that stimulation of striate cortex and abutting extrastriate V2 results in similar frequencies of positive phosphene reports. We also found that the shapes of phosphenes were very similar in the phosphenes elicited by V1 and V2 stimulation. By comparison, Lee et al. (2000) reported that more complex geometric shapes of phosphenes (e.g., triangles, diamonds) are produced via electrodes placed on the extrastriate cortex compared with the phosphenes produced from the striate cortex stimulation. Yet, our results imply that stimulation of the extrastriate area V2 does not induce more complex geometric shapes compared with the striate cortex stimulation. It is noteworthy that Lee and coworkers did not identify the functional visual areas with functional imaging, and thus it is not clear which visual areas were actually stimulated in their experiment. It could be anticipated that more complex shapes of phosphenes were produced by the stimulation of higher visual areas (e.g., V3, V3a, lateral occipital cortex) than V2. However, the results by Murphey et al. (2009) are not in line with this interpretation as they targeted the stimulation to the functionally defined V3, lateral occipital cortex, V4, V8, MT, parahippocampal place area, and fusiform face area, but did not find that the characteristics of phosphenes would be more complex in these areas than in V1/V2. Thus, the relationship between phosphene complexity and different visual areas remains unclear.

In this study, 2 participants reported chromatic phosphenes which was expected as V1 and V2 respond to chromatic information (e.g., Hubel and Wiesel 1968; Baizer et al. 1977;
Livingstone and Hubel 1984; Ts’o and Gilbert 1988; Kleinschmidt et al. 1996; Engel et al. 1997). We also found that the chromatic phosphenes contained more unique hues (red, yellow, orange, brown, and blue) than the other hues (orange, brown, or purple). Two channels (red-green, L-M, and yellow-blue, S-[L + M]) carrying wavelength information ascend to V1 from the lateral geniculate nucleus. Although it is not well known how chromatic information is represented in the visual cortex, single-cell recordings in monkeys (De Valois et al. 2000) and fMRI studies in humans (Goddard et al. 2010) suggest that as early as in V1, neurons combine the input from the 2 channels. Yet, our results suggest that the critical part of the neural processes underlying subjective experience of the hues of brown, orange, and purple lie in the color-sensitive visual areas higher than V1 or V2 (Lueck et al. 1989), named in different studies as hV4 (Wade et al. 2002), VO-1, and VO-2 (Brewer et al. 2005) or V8 (Hadjikhani et al. 1998). Alternatively, V1 and V2 may contain significantly less neurons sensitive to the hues brown, orange, and purple compared with neurons sensitive to unique hues. In our study, of all 800 phosphenes, there were no phosphenes with the color orange, but there were a few phosphenes with the color purple. The result is in line with the finding by Goddard et al. (2010) who showed that there is a greater response for lime-magenta than orange-cyan stimuli across cortical visual areas. However, given that our data concerning chromatic phosphenes is based on reports from only 2 participants, future studies should further explore these questions in larger groups of participants. It is possible that some of the statistical tests that showed just a trend in the present study would become statistically significant (or clearly nonsignificant) with larger number of participants. Yet, it should be noted that selective stimulation is possible only for carefully selected participants, which requires relatively large human and financial resources. For instance, only half of the participants from the larger group of 10 participants were suitable for the present study on the bases of their functional topography of V1 and V2.

Interestingly, the results also showed that the phosphenes tended to appear at a larger eccentricity than the target area (Supplementary Data). Thus, it is possible that the phosphenes were not generated solely in the area in which the E-field strength was strongest, but that TMS induced a spreading neural activation elsewhere which in turn induced the subjective experience of phosphenes. Support for this view comes from the observation that during V2 stimulation some of the phosphenes crossed the horizontal midline (see Supplementary Fig. S10). Ventral and dorsal V2 are located separately in the cortex, relatively far from each other. Most of the subareas of dorsal V2 are located quite close to the surface of the brain whereas the ventral V2 (and also the retinotopically homologous V1) is located deeper in the brain and is not easily accessible with TMS. When dorsal V2 was stimulated, it could be predicted that the phosphenes should have a clear cut off point at the horizontal meridian. However, this was not the case. In addition, there were visual field regions in which the participants reported phosphenes in more than 50% of trials, but the E-field strength in the cortical representation of those areas was below 50% of the phosphenes threshold. An interpretation that horizontal connections within a V1 or V2 are activated in phosphenes does not get support from the results of the studies done with nonhuman primates which demonstrate that cortical activation due to microstimulation of V1 can spread only couple of millimeters (Tolias et al. 2005; see also Gilbert and Wiesel 1989; Angelucci et al. 2002; Tehovnik et al. 2006). That distance is probably not sufficient (Angelucci et al. 2002) to explain the large eccentricities of phosphenes observed in the present study. Instead, the TMS may activate a rapid feedforward-feedback-pathway, which is associated with surround modulation of classical receptive field responses in the V1 in primates (Angelucci and Bressloff 2006; Schwabe et al. 2006, 2010), including far surround (Ichida et al. 2007). Yet, it is not clear whether the cells that represent peripheral visual field regions trigger action potentials or would it be possible that when there are no rival signals, local receptive fields would represent a larger visual field region in perception.

The interpretation that the neural connections from the TMS-targeted cortical area to the other visual areas contribute to the generation of phosphenes is supported by some earlier studies. In macaque, microstimulation of V1 induces activation in V2, V3, and MT (Tolias et al. 2005). Furthermore, the study by Pascual-Leone and Walsh (2001) suggested that in humans, the feedback activation from V5 to the early visual areas is necessary for the sensation of movement of TMS-induced phosphenes. In line with this, as mentioned above, a study of a patient with V1 lesion demonstrated that TMS of an extrastriate area ipsilateral to V1 lesion did not induce phosphenes (Cowey and Walsh 2000). However, a later study showed that when both V5 areas were stimulated simultaneously, phosphenes were produced also in the contralateral visual field to the damaged V1 which implied that particularly the feedback projections from the extrastriate areas to ipsilateral V1 are probably not a prerequisite to induce phosphenes (Silvanto et al. 2007). In contrast, to produce visual sensations, extrastriate areas seem to be dependent on the interconnected visual areas in a more complicated manner (Silvanto 2008). Consequently, also in the present study, it remains open whether V1 or extrastriate areas, such as dorsal V3, would be involved via cortico-cortical connections in the generation of phosphenes during V2 stimulation, or whether extrastriate areas would be involved in the phosphenes generated by V1 stimulation. Moreover, it is not clear why phosphenes tend to appear in the peripheral vision. One possibility is that, given that a smaller neuron group represents peripheral vision than focal vision, the cell group that represents peripheral vision crosses the threshold more easily during artificially induced spreading of activation than does the neuron group that represents focal vision. This question remains to be solved in future studies.

In summary, the present study provides strong causal evidence that stimulation of V1 and V2 are equally capable of generating subjective light sensations, as demonstrated by comparable phosphenes thresholds. Nevertheless, brightness ratings of phosphenes show that with equal stimulation of V1 and V2, the activation of V1 makes a stronger contribution to the intensity of the basic experience of light.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

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