

Neuronavigated transcranial magnetic stimulation suggests that area V2 is necessary for visual awareness

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ABSTRACT

The primary visual cortex (V1) has been shown to be critical for visual awareness, but the importance of other low-level visual areas has remained unclear. To clarify the role of human cortical area V2 in visual awareness, we applied transcranial magnetic stimulation (TMS) over V2 while participants were carrying out a visual discrimination task and rating their subjective awareness. Individual retinotopic maps and modelling of the TMS-induced electric field in V1, V2 and V3d ensured that the electric field was at or under the phosphene threshold level in V1 and V3d, whereas in V2 it was at the higher suppressive level. As earlier shown for the V1, our results imply that also V2 is necessary for conscious visual experience. Visual awareness of stimulus presence was completely suppressed when the TMS pulse was delivered 44–84 ms after the onset of visual stimulus. Visual discrimination and awareness of stimulus features was impaired when the TMS pulse was delivered 44–104 ms after the visual stimulus onset. These results suggest that visual awareness cannot be generated without an intact V2.

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1. Introduction

Finding the neural mechanisms necessary for the subjective experience of seeing, visual awareness, is one of the major tasks in solving the problem of consciousness. The evidence supports the idea that primary visual cortex (V1) is necessary for visual awareness, because the removal of V1 induces perceptual blindness in primates, and partial lesions of the human V1 are associated with scotomas (Holmes, 1918; for review see Tong, 2003). The evidence for the necessity of V1 for visual awareness does not imply that V1 directly generates visual awareness because it may simply be a distributor of neural input to higher-level areas that participate in the actual generation of visual awareness more directly than V1 (Crick & Koch, 1995). Alternatively, V1 may form a subcomponent in a larger recurrent network whose activity generates visual awareness (Lamme, 2004).

While the role of V1 in visual awareness has been extensively studied, the role of the adjacent area V2 for visual awareness remains unknown. V1 and V2 are densely connected with each

other, which suggests that also V2 might play a role necessary for the generation of visual awareness. In primates, extensive bidirectional connections link V1 and extrastriate area V2, and while most connections from V1 reach other extrastriate areas via V2, some of the direct connections from V1 terminate in higher visual areas (e.g. V3, V3a, V4 and V5) without a relay via V2 (e.g. Burkhalter, Felleman, Newsome, & Van Essen, 1986; Felleman, Burkhalter, & Van Essen, 1997; Felleman & Van Essen, 1991; Maunsell & Van Essen, 1983; Van Essen, Newsome, Maunsell, & Bixby, 1986). The aim of the present study was to explore the role of V2 in visual awareness in humans.

Regarding the role of V2 in visual perception, the results from the single unit recordings in non-human primates show that neurons in V2 are selective to the simple features of stimuli such as size, orientation, colour and direction (e.g. Gegenfurtner, Kiper, & Fenstemaker, 1996; Hubel & Livingstone, 1987) and also to more complex shapes such as star, spiral, angle, etc. (Hegd  & Van Essen, 2000; Hegd  & Van Essen, 2003; Hegd  & Van Essen, 2007). Furthermore, neuroimaging studies in humans and single cell recordings in monkeys have consistently associated processing of illusory contours to activation in V2 (e.g. von der Heydt, Peterhans, & Baumgartner, 1984; for review see Seghier & Vuilleumier, 2006). While typical neuroimaging studies can reveal which brain areas' activity correlates with visual perception and awareness, only

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studies that interfere with the normal function of brain areas can reveal whether the activation of the area causally contributes to visual awareness and is thus necessary for it. For the causal role of V2 in visual perception, Merigan, Nealey, and Maunsell (1993) demonstrated that a lesion of V2 in a monkey impairs complex orientation discrimination, but it does not have an effect on visual acuity or contrast discrimination in relatively simple tasks, such as discriminating between horizontal and vertical gratings. In humans, transcranial magnetic stimulation (TMS) over V2 combined with TMS-induced electric field (E-field) modelling imply that V2 is necessary at least for discrimination of orientation of U-shaped hook (Thielscher, Reichenbach, Uğurbil, & Uludağ, 2010). However, Thielscher et al. (2010) and most of the other studies on the role of V2 in visual perception have measured behavioural forced-choice responses, not subjective awareness. Given that discrimination of visual stimulus may occur above chance level without conscious experience of the stimulus, for example as demonstrated by blindsight patients (e.g. Pöppel, Held, & Frost, 1973; Trevethan, Sahraie, & Weiskrantz, 2007; Weiskrantz, 1997; Weiskrantz, Warrington, Sanders, & Marshall, 1974), behavioural forced-choice responses do not necessarily reflect contents of visual awareness.

TMS over the occipital cortex interferes with visual perception (e.g. Amassian et al., 1989; Corthout, Uttl, Ziemann, Cowey, & Hallett, 1999; Epstein & Zangaladze, 2001; Kastner, Demmer, & Ziemann, 1998; Pascual-Leone & Walsh, 2001; Paulus, Korin, Wischer, & Tergau, 1999), but the methodology of the earlier TMS studies on visual awareness has not differentiated V1, V2 and V3 stimulation. In contrast to the commonly used spatially coarse TMS methodologies involving TMS targeting without retinotopic maps and modelling of induced E-field, recent methodological studies (Salminen-Vaparanta, Noreika, Revonsuo, Koivisto, & Vanni, 2012; Thielscher et al., 2010) have established that a sufficient accuracy can be achieved in targeting the TMS pulse to the specific early visual area. Furthermore, by comparing different positions of the figure-of-eight coil on the scalp with the response accuracy in orientation discrimination task, Thielscher et al. demonstrated that the spatial resolution of TMS can be as good as 7 mm.

It is well established that the lowest TMS-induced E-field strength required to produce visual suppression is higher than the lowest E-field strength required to induce phosphenes, that is, fleeting sensations of light (e.g. Abrahamyan, Clifford, Arabzadeh, & Harris, 2011; Kammer, 1999; Kammer, Puls, Erb, & Grodd, 2005; Kastner et al., 1998; Thielscher et al., 2010). In particular, Abrahamyan et al. (2011) tested the effect of E-field strength slightly under the individual phosphene threshold (defined as the level where phosphenes were experienced with 60% probability) on visual detection threshold and found that the E-field strength under the phosphene threshold improves visual detection. The E-field strength just at the phosphene threshold did not have an effect on visual detection (Abrahamyan et al., 2011). In our earlier study, we showed that when the E-field strength was increased by 20% above the phosphene threshold intensity (defined as experiences of phosphenes with 50% probability), visual suppression occurred (Salminen-Vaparanta et al., 2012). In addition, Thielscher et al. (2010) demonstrated that by decreasing the TMS stimulator output intensity by about 15% from the suppressive level, the accuracy of the responses increased from the chance level to 100% correct. Thus, by decreasing the stimulator output intensity visual suppression declines fast, and the stimulation at the phosphene threshold intensity is too weak to produce suppression. These observations are useful in studying the effects of TMS on a specific early visual area: In order to induce visual suppression due to TMS on the target area, the estimated E-field strength in the non-target adjacent areas should be kept under the level required to induce suppression (e.g. at the phosphene threshold level or below). This procedure enables

unravelling of latencies, separately in different early visual areas (Salminen-Vaparanta et al., 2012; Thielscher et al., 2010).

In the current study, we combined neuronavigated TMS with a computational model of the TMS-induced E-field to test whether or not visually evoked activation in V2, like in V1, is necessary for visual awareness. If interference with the normal function of V2 precludes visual awareness of stimuli, then V2 plays a necessary causal role in producing visual awareness of stimuli. This would be consistent with human data suggesting that extrastriate lesions can cause homonymous visual field defects (Horton & Hoyt, 1991; Slotnick & Moo, 2003). The contrary hypothesis states that interference of V2 does not preclude the visual awareness of stimuli, because the connections from V1 to higher areas that bypass V2 provide sufficient input for the generation of visual awareness. The aim of this study was to test which of these two opposite hypotheses receives more empirical support when V2 activity is disrupted by TMS.

2. Materials and methods

2.1. Participants

Nine participants were recruited for the experiment. Participants signed the informed consent and they were treated in accordance with the Declaration of Helsinki. The experiment was approved by the ethical committee of the Hospital District of Southwest Finland. One participant was rejected because the TMS-induced intracranial E-field strengths in her V1 and V2 were similar, rendering V2 stimulation unselective. Another participant was excluded from the analyses because he continually reported seeing attention-capturing phosphenes during the TMS experiment. We report here the results from seven healthy participants (21–27 years, 2 males) with normal or corrected-to-normal vision. The participants were not aware of the purposes of the study apart from one of the participants (author VN, who was still blind to the experimental conditions).

2.2. Functional magnetic resonance imaging

By using multifocal functional magnetic resonance imaging (fMRI), twenty-four retinotopic subareas in the V1 and V2 (Fig. 1A) were determined for each participant (for details, see Salminen-Vaparanta et al., 2012; Vanni, Henriksson, & James, 2005). Presentation™ software (Neurobehavioral Systems Inc., Albany, California, USA) with data projector (Christie X3™, Christie Digital Systems Ltd., Mönchengladbach, Germany) was used to present the visual stimuli (extending from 1° to 12° eccentricity). The data was acquired with a 3T MRI scanner (Signa™ Excite, General Electric Inc., WI, USA), with a phased-array 8-channel head coil. The parameters for the single shot gradient-echo echo-planar imaging sequence in functional imaging were: repetition time 1800 ms, echo time 30 ms, matrix 64 × 64, flip angle 60°, FOV 160 × 160, and slice thickness 2.5 mm. High-resolution structural images were obtained with SPGR sequence, with matrix 256 × 256, FOV 250 × 250, and slice thickness 0.9 mm.

The fMRI data were analysed with the SPM2 toolbox (Wellcome Department of Imaging Neuroscience, London, UK). SPM t-maps were overlaid on an anatomical 3D image, and the borders between the V1, V2 and V3 were identified. Separate representations for each of the regions (subareas) were determined with SPM2, and corresponding coordinates of the centre of the target V2 subarea and the retinotopically corresponding V1 and V3d subareas were selected for the later TMS targeting in eXimia Navigated Brain Stimulation (NBS) (Nexstim Ltd., Helsinki, Finland) software. The most optimal subarea for V2 stimulation was selected according to individual functional anatomy of early visual areas, and the visual stimuli during the TMS experiment were presented in the retinotopically corresponding visual field location. The target subarea was in the right hemisphere for three participants and in the left hemisphere for four participants (regions 6 and 8, respectively, see Fig. 1A). The mean distance between the approximate centres of V1 and V2 subareas was 11 mm.

2.3. TMS and E-field calculation

eXimia™ TMS magnetic stimulator (Nexstim Ltd.) was used with a figure-of-eight Nexstim bipulse coil (outer winding diameter = 70 mm). The coil was held tangentially against the participant's head with the coil holder, and head position was stabilised with a chin rest. MRI-guided NBS system (eXimia 2.1.1; Nexstim Ltd.) was applied to provide the calculation of the TMS-induced E-fields and accurate 3-D localisation of the coil in relation to the brain. The current direction of the second phase of the biphasic pulse was from the lateral to the medial (Corthout, Barker, & Cowey, 2001; Kammer, Vorwerk, & Herrnberger, 2007; Fig. 2A). The spatial relation between the brain and the TMS-induced E-field was continuously registered by the NBS system with a 5.7 mm spatial resolution containing all sources of errors (i.e. registration to anatomical MRI's, manufacturing tolerances, optical tracking, E-field

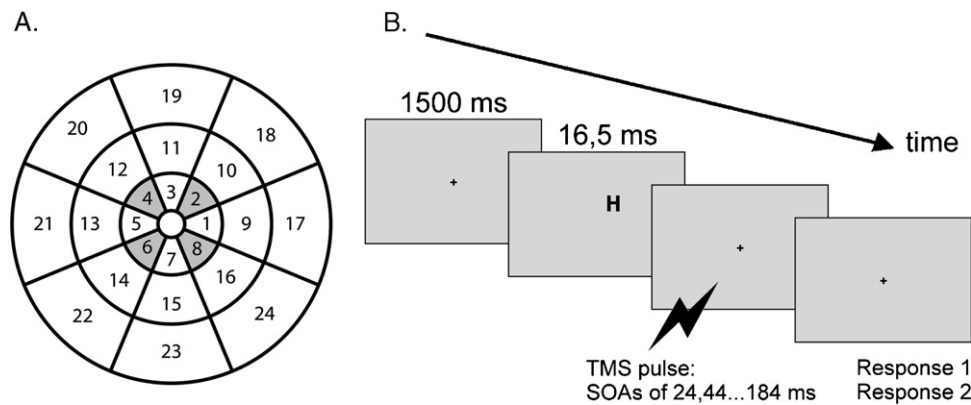


Fig. 1. (A) Multifocal fMRI design allowed parallel mapping of 24 visual field representations in V1 and V2. The three rings extended on the visual field at 1–3.2°, 3.2–6.7°, and 6.7–12° eccentricities. The TMS targets were the cortical representations for visual field regions 6 or 8. Contralateral representations for the upper visual field, regions 2 or 4, served as control sites. (B) The temporal structure of a single trial. In a trial, a fixation cross (diameter 0.25°) was presented on the screen, followed by one of the letter stimuli (2.8° away from fixation), after which fixation cross was presented again until the participant had given the responses. The TMS pulse was applied in a random order at one of the nine visual stimulus–TMS pulse SOAs.

computational model and movements of the head tracker during the stimulation (Ruohonen & Karhu, 2010)).

The TMS-induced E-field distribution in V2 and in the retinotopically corresponding V1 and V3d was modelled with the spherical conductor model (Heller & van Hulsteyn, 1992; Sarvas, 1987). The model was used to estimate the E-field strength at the phosphene threshold intensity (Section 2.4) and during the behavioural suppression experiment (Section 2.5). The calculation of the TMS-induced E-field is based on the shape of the TMS coil, the position and orientation of the coil in relation to the brain, the electrical conductivity properties of the tissue (Ilmoniemi, Ruohonen, & Karhu, 1999) and the local variation in the head curvature of each individual. It is assumed that TMS excites the cortex in an orientation-selective manner in a way that the optimal direction of the TMS-induced E-field to induce depolarisation in neurons is perpendicular to the sulcal banks (Fox et al., 2004). However, the spherical conductor model does not account the conductivity differences caused by the gyral folding. In the current study, the targeted V2 subareas and nontargeted V1 and V3d subareas (with a few exceptions) contained neurons in various orientations in relation to the E-field. This was due to the strong gyral folding of visual cortex in relation to the size of the stimulated subareas (diameter about 5–15 mm) and the focal area of the stimulation hotspot (about 0.68 cm² or more).

The suitability of the spherical model for the occipital lobe stimulation has been discussed in more detail earlier (Hämäläinen & Sarvas, 1989; Salminen-Vaparanta et al., 2012; Thielscher et al., 2010). In brief, recent finding comparing the results of spherical model with those of finite element model suggests higher focality of E-fields as a product of more realistic model, especially in the gyral crowns (Thielscher, Opitz, & Windhoff, 2011). In addition, the boundaries inside the skull modify the E-field strength values (Opitz, Windhoff, Heidemann, Turner, & Thielscher, 2011; Thielscher et al., 2011). Nevertheless, the nearly spherical shape of the posterior part of the head reduces the absolute value of electrical conductivity, especially when the individual local variation in the shape of the head is taken into account (Davey, 2008; Ruohonen & Karhu, 2010) and regions close to the head surface are targeted (Hämäläinen & Sarvas, 1989). Thus, a more realistic model than the spherical model might provide more accurate values of E-field distribution in the early visual cortex in the current study, but for the purposes of the present study (to target specific early visual area) the spherical model can be regarded sufficiently accurate. The mean error in E-field hotspot location, emerging from the E-field modelling procedure, is about 3.8 mm (Ruohonen & Karhu, 2010). This modelling includes intracranial E-field and coil's properties, and fitting of the model to individual's head (Ruohonen & Karhu, 2010). Note that this value is also included in the estimated spatial resolution of NBS system.

2.4. TMS intensity

To ensure that V2 was the primary target of stimulation rather than V1, we modelled the TMS-induced E-field in V2 and in the retinotopically equivalent V1. The E-field strength was estimated in the approximate centre of each selected subarea. We set two criteria for selecting the intensity of the TMS stimulation. First, TMS intensity had to be at the visual suppression level in the targeted V2 subarea. Second, we aimed to select an intensity that would induce lower E-field strength in the retinotopically equivalent V1 than the E-field strength at 120% of the individual phosphene threshold. This criterion was based on the results of the earlier study (Salminen-Vaparanta et al., 2012), in which we directed the TMS pulses to V1 while the participants carried out an experimental task similar to the one in the present study. When the E-field strength was increased by 20% above the phosphene

threshold intensity, visual suppression occurred with about 40% drop off of accuracy in a forced-choice task.

The phosphene threshold (i.e. the stimulation intensity that induces phosphenes with a 50% probability (Kammer, Beck, Erb, & Grodd, 2001)) was determined for each individual participant by directing the TMS pulse to the V1 subarea (Salminen-Vaparanta et al., 2012) which retinotopically corresponded to the selected V2 subarea. Maximum likelihood threshold hunting (MLTH) procedure (Awiszus, 2003) was applied defining the phosphene thresholds. However, this procedure was not applicable for three participants who reported phosphenes only with intensities around the phosphene threshold but who did not report phosphenes with higher stimulation intensities, which is required for MLTH procedure. For these participants the phosphene threshold was determined by delivering pulses in a randomised order in steps of one percentage unit between 30% and 55% of the stimulator output intensity. During the phosphene threshold determination, for 5/7 participants the E-field strength was higher in the selected V2 subarea than in the retinotopically corresponding V1. Thus, for these five participants the primary source of phosphenes was most probably V2. For the area in which the E-field strength was highest (selected V2 subarea or retinotopically equivalent V1 subarea), we estimated the E-field strength corresponding to the phosphene threshold.

For the actual visual discrimination experiment (see Section 2.5), the stimulator output intensity was set to 120% of the individual phosphene threshold intensity, rendering 55% of the maximal stimulator output as the mean intensity for the experiment (SD = 5.7, min = 44%, max = 63%). When V2 was the target of stimulation, this intensity level ensured that the E-field strength was higher for all participants in V2 than in retinotopically corresponding V1 subarea (mean difference 32%, Fig. 2). In particular, for 4/7 participants the E-field strength in V1 was below the phosphene threshold intensity. It can be assumed that for these four participants there may have been, on the one hand, improvement of visual sensitivity due to TMS of V1 (Abrahamyan et al., 2011) and, on the other hand, the suppressive effect from stimulation of V2. For one of the participants the E-field strength in V1 was at the same level with the phosphene threshold intensity, in which case no effect on visual sensitivity is expected due to TMS of V1 (Abrahamyan et al., 2011), but only the suppressive effect due to TMS of V2. For two participants (P5 and P7), the E-field strength in V1 was higher than the E-field strength at the phosphene threshold intensity, but still less than 120% of the phosphene threshold intensity and considerably lower than the E-field strength in V2. In these two cases, we cannot exclude the possibility that the stimulation of V1 may have affected the results by producing some visual suppression and thus we repeated the statistical analyses also without these two participants (see Section 3). Altogether, given that visual suppression threshold is higher than the phosphene threshold (e.g. Abrahamyan et al., 2011; Kammer, Puls, Erb, et al., 2005), we are confident that TMS-induced suppression of visual awareness in the main experiment was due to the disturbance of visual processing in V2 rather than in V1.

In addition, we identified the strength of the TMS-induced E-field during the main experiment (Section 2.5) in the retinotopically corresponding V3d (n = 5). The E-field strength values in V3d were 84 V/m for participant P1, 137 V/m for P2, 129 V/m for P3, 131 V/m for P6 and 94 V/m for P7. For four participants the E-field strength was under or at the phosphene threshold intensity whereas for one participant (P7) it was higher than the E-field strength at the phosphene threshold but still lower than the E-field strength in V2. For two participants, the fMRI failed to evoke reliable responses for the delineation of V3d, and thus it was not possible to define the E-field strength in V3d for these participants.

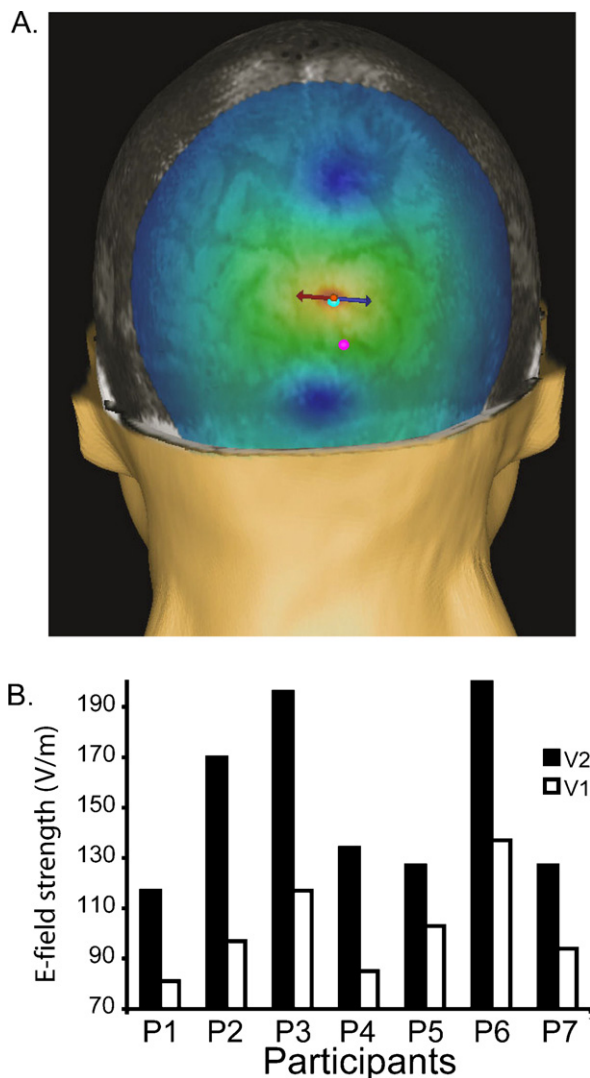


Fig. 2. (A) The E-field distribution demonstrated in the occipital cortex of one representative participant. The orange spot marks the location of the centre of the coil and the colours blue–green–yellow–red show the E-field strength in an increasing order. The direction of the E-field of the second phase of the biphasic pulse is shown by the red arrow and the E-field direction of the first phase by the blue arrow. The blue spot marks the centre of target V2 subarea, and the pink spot shows the centre of retinotopically equivalent V1 subarea. (B) Averaged E-field strength in V2 and in retinotopically equivalent V1 in all participants (P), when TMS pulses were targeted to V2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.5. Behavioural task and experimental procedure for TMS

Three dark grey letters (H, T, O, diameter 0.23°) were used as visual stimuli and they were presented on a light grey background (31 cd/m²) on a 19" monitor (60 Hz; 16.5 ms per frame) with Presentation™ software. Viewing distance was 90 cm. Visual stimuli and behavioural tasks were similar to the earlier study from the same laboratory (Salminen-Vaparanta et al., 2012). Stimuli were presented for 16.5 ms in a randomised order in the lower or upper visual field. In each trial (Fig. 1B), the participants were asked to identify the letter stimulus and to evaluate their visual experience of the stimulus according to the scale: (1) I saw the stimulus clearly, that is, I saw at least a feature of the letter from which I could recognise it, (2) I did not see the stimulus clearly, but I saw a trace on the screen, or (3) I did not see anything at all, only the fixation point. The participants gave keyboard responses for the forced-choice letter-discrimination task with three fingers of the right hand, and for the subjective rating with three left hand fingers.

When the subarea in the right hemisphere was stimulated with TMS, the visual stimuli were presented in a randomised order either in the retinotopically corresponding lower left visual field or in the opposite upper right field (Fig. 1A). Alternatively, when the subarea in the left hemisphere was stimulated, the visual stimuli were presented either in the retinotopically corresponding region

in the lower right field or in the opposite region in the upper left field. As the cortical representation of the upper visual field was not stimulated, the performance on upper visual field stimuli was used as control condition. Thus, the nonspecific physical effects of TMS were exactly the same in the control condition and in the main experimental condition. The contrast level of the visual stimuli was determined individually in pre-experimental stimulus blocks (30 trials/block) by systematically increasing or decreasing the contrast level between the blocks to reach 75–85% discrimination accuracy without TMS (mean luminance of the visual stimulus 8 cd/m²; range = 0.18–19.07; 99%–38% Weber contrast, respectively).

There were 48 trials for each visual field position ($N=2$) at each visual stimulus–TMS pulse onset-asynchrony (SOA) from 24 ms to 184 ms in steps of 20 ms ($N=9$), resulting in altogether 864 experimental trials for each participant. The experiment was divided into 16 TMS blocks (54 trials in each block). From the total of 112 blocks, in two participants, three blocks had to be removed from the data analyses due to the movements of the head tracker during the TMS experiment or some other technical error. In addition, between the TMS blocks each participant carried out four baseline blocks (54 trials each) without TMS.

3. Results

To analyse how different SOAs (9: 24–184 ms) and visual fields (2: upper, lower) affected response accuracy and ratings of subjective awareness, we carried out repeated measures analyses of variance (ANOVA) with Huynh–Feldt correction applied to p -values. Post hoc analysis comprised paired samples t -tests to compare results at each SOA between TMS and no TMS baseline conditions in the corresponding visual field. In these comparisons, Benjamini and Hochberg's linear step up procedure (Benjamini & Hochberg, 1995) was applied to reject possible false positives due to multiple comparisons. Visual field ($F_{(1,6)}=24.58$, $p=.003$) and SOA ($F_{(8,48)}=7.30$, $p<.001$) influenced letter discrimination accuracy and interacted with each other ($F_{(8,48)}=3.62$, $p=.026$; Fig. 3A). TMS over V2 did not impair discrimination of the upper visual field stimuli (the cortical representation of which was not stimulated), but the response accuracy was impaired for the lower visual field stimuli at SOAs from 44 to 104 ms when compared to the no TMS baseline condition (p -values <0.05).

For the analysis of awareness of stimulus presence, the dependent measure was the percentage of trials in which the participants reported either having seen the stimulus clearly (the first response option) or having seen just a trace of the stimulus (the second response option). In the rest of the trials the participants did not see the stimulus at all. The results showed, as illustrated in Fig. 3B, that when the TMS pulses were delivered to V2 within 84 ms after visual stimulus onset, participants frequently reported that they did not see anything appearing on the screen. This was confirmed by the statistical analysis which showed the main effects for visual field ($F_{(1,6)}=21.23$, $p=.004$) and SOA ($F_{(8,48)}=8.84$, $p=.002$) and also an interaction effect ($F_{(8,48)}=5.99$, $p=.004$). Awareness of stimulus presence was impaired for the lower visual field stimuli from 64 to 84 ms post stimulus (p -values <0.05). TMS did not impair visual awareness of stimuli presented to the upper visual field, confirming that visual suppression was not due to nonspecific effects of TMS, such as attention lapses caused by muscle contractions, auditory clicks or involuntary blinks.

In addition, we analysed awareness of stimulus features which was operationalised as the percentage of trials in which participants reported that they saw clearly at least some feature of the letter stimulus. Thus, in the rest of the trials participants reported seeing either something or nothing at all on the screen. As illustrated in Fig. 3C, TMS suppressed participants' awareness of stimulus features for the stimuli presented to lower visual field. In particular, SOA ($F_{(8,48)}=5.64$, $p=.003$) and visual field ($F_{(1,6)}=7.85$, $p=.031$) showed main effects and also interacted mutually ($F_{(8,48)}=5.11$, $p=.020$). TMS diminished awareness of stimulus features in the lower visual field stimuli at SOAs from 44 to 104 ms (p -values <0.05) when compared with the no TMS baseline

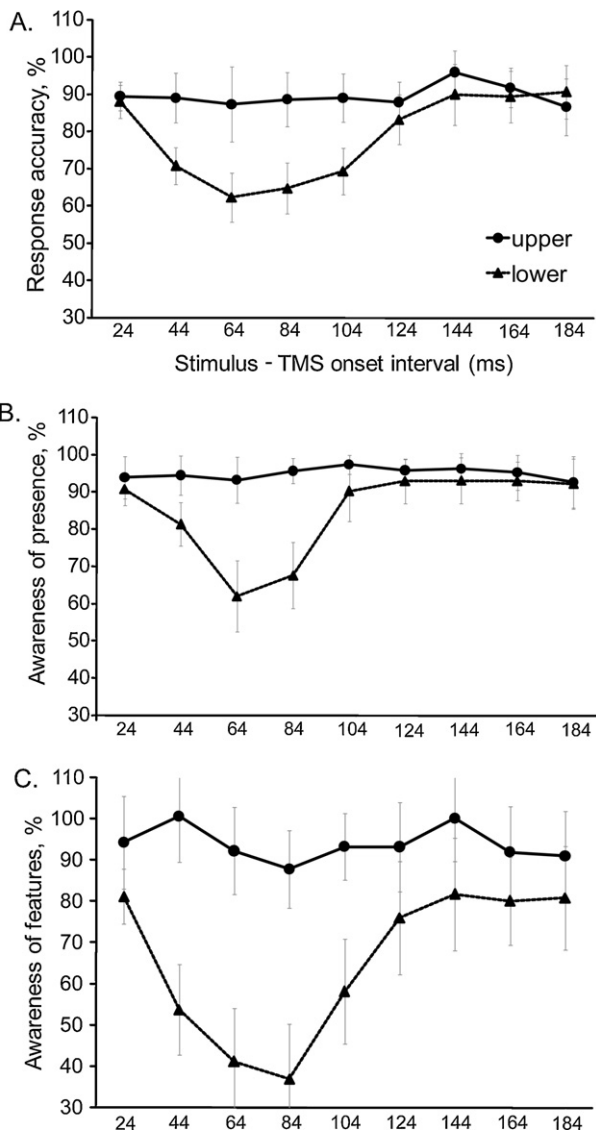


Fig. 3. (A) The letter discrimination accuracy, (B) awareness of stimulus presence, and (C) awareness of stimulus features when TMS pulses were directed to the lower visual field representation in V2 and visual stimuli were presented either to the lower (TMS targeted) or to the upper (control) visual field region. The results ($N = 7$) are scaled so that 100% represents the baseline performance without TMS. Error bars indicate standard error of the mean. The latencies when TMS decreased statistically significantly recognition accuracy and awareness in relation to no TMS baseline are given in the main text.

condition. TMS did not affect visual awareness of stimulus features in the upper visual field.

To eliminate the impact of two participants (P5, P7) whose E-field strength in V1 exceeded the phosphene threshold we run the analyses also without these two participants. In this subgroup, for 4/5 participants the E-field strength in V3d was at the phosphene threshold level or under it. TMS diminished awareness of stimulus features at the same SOAs (p -values $< .025$) as in the group of all seven participants and awareness of stimulus presence at SOAs from 44 to 84 ms post stimulus (p -values $< .025$). However, while visual field and SOA showed an interaction effect for awareness of stimulus presence ($F_{(8,32)} = 10.10$, $p = .008$) and features ($F_{(8,32)} = 7.37$, $p = .010$), the analysis of letter discrimination accuracy showed only main effects for visual field ($F_{(1,4)} = 18.01$, $p = .013$) and SOA ($F_{(8,32)} = 9.90$, $p < .001$), but did not reveal an interaction effect.

In additional control sessions, two participants with optimally located V1 subareas carried out the same behavioural experiment while their V1 was stimulated (Supplemental Materials). Given that the decline of visual perception is stronger if TMS-induced E-field strength is increased (Kammer, Puls, Strasburger, Hill, & Wichmann, 2005), we hypothesised that if actually V1, not V2, is the site of visual suppression in the main experiment, then by increasing the TMS-induced E-field strength in V1 (and decreasing the E-field strength in V2), the magnitude of visual suppression should increase. However, as illustrated in Supplemental Materials, Fig. S1, the visual suppression was not stronger during the V1-targeted than during the V2-targeted stimulation. Instead, the results suggest longer V2 peak latencies than V1 latencies in the neural processes that are necessary for visual awareness (Fig. S1). The equal magnitude of visual suppression in stimulation of V1 and V2 and the longer involvement of V2 in visual suppression are consistent with the interpretation that the suppression of visual awareness in the main experiment was due to the effects of TMS on V2 rather than on V1.

4. Discussion

In the present study, the possible contribution of V2 in the generation of visual awareness was investigated. The main result showed that TMS targeted to V2 suppresses awareness of stimulus presence. Importantly, we ensured that the suppression was not produced by accidental stimulation of V1 or V3d by modelling the TMS-induced E-field in V2 and in the retinotopically equivalent V1 and V3d. When V2 was the target, the E-field strength was higher in V2 than in V1 for all the participants. Furthermore, for five participants, the E-field strength in the retinotopically corresponding V1 subarea was lower or at the same level as the E-field strength at the phosphene threshold intensity, and for four of these participants the same applied for V3d. These participants showed significant suppression of visual awareness of stimulus presence when V2 was stimulated. Noteworthy, the E-field strength under the phosphene threshold facilitates visual detection, whereas at the phosphene threshold it has no effect on visual detection (e.g. Abrahamyan et al., 2011). Thus, when our results are interpreted according to the results of Abrahamyan et al. (2011), the stimulation of V2 in the present study was not selective in the sense that V1 or V3d were not affected at all, but the E-field strength was inhibitory in V2 whereas it was either facilitatory or neutral in V1 and V3d. Altogether, the results support the view that intact V2 functioning is necessary for visual awareness. Conversely, the cortical connections from V1 to the extrastriate areas (e.g. V3, V3a, V4, and V5) bypassing V2 are probably not sufficient for conscious detection of stimulus presence.

The first responses to visual stimuli in V1 occur approximately 40–60 ms after the stimulus onset in humans (e.g. Clark, Fan, & Hillyard, 1995; Vanni et al., 2004; Wilson, Babb, Halgren, & Crandall, 1983) and V2 is activated only a few milliseconds later (Nowak, Munk, Girard, & Bullier, 1995; Raiguel, Lagae, Gulyàs, & Orban, 1989). Interestingly, TMS over V2 interfered with awareness of stimulus presence as late as 84 ms after the stimulus onset in the present study, which implies that the earliest neural signals from the striate cortex via V2 (Nowak et al., 1995) to the higher areas (within about 80 ms from the stimulus onset) are not sufficient to generate visual awareness. There are at least two alternative explanations for the critical role of V2 in awareness of stimulus presence. First, given that neurons in V2 are selective to simple shapes of stimuli (e.g. Gegenfurtner et al., 1996; Hubel & Livingstone, 1987), V2 might be important for awareness in terms of local visual information processing in V2. Another plausible explanation is that the feedback from V2 to V1 is important to visual awareness by

modulating the later feedforward activation from V1 to the higher areas and backward projections to subcortical structures. While the effect of inactivation of V2 on neurons in V1 is not clearly established (Hupé, James, Girard, & Bullier, 2001; Sandell & Schiller, 1982; see also, Salin & Bullier, 1995), responses in monkey's V1 are modulated by the feedback from V2 at least to some extent (Bullier, Hupé, James, & Girard, 1996), and V1 receives the majority of its cortical top-down projections from V2 (Barone, Batardiere, Knoblauch, & Kennedy, 2000). However, V1 stimulation with two participants (Supplemental data) did not give support for this interpretation as the suppression of awareness continued slightly longer after V2 stimulation than after V1 stimulation. No such delay would be expected if the feedback projection from V2 to V1 would be necessary for awareness. Instead the longer peak latencies for V2 than for V1 suggest that for these two participants there was feed-forward activation that was necessary for awareness. This could be re-tested in future studies with a larger sample of participants by comparing the duration of visual suppression after V1 and V2 stimulation.

The results showed that letter discrimination is impaired if TMS pulse is delivered to V2 between 44 and 104 ms after visual stimulus onset. This finding is consistent with earlier reports that a lesion of V2 in a monkey (Merigan et al., 1993) and the TMS stimulation of human V2d (Thielscher et al., 2010) impairs orientation discrimination, implying that activation of projections that bypass V2 are not sufficient for more complex orientation discrimination. Moreover, letter discrimination performance and awareness of stimulus features were affected by the TMS in the same time window (44–104 ms), which is reasonable because both processes require visual processing of the stimulus details. Note that the contribution of V2 was necessary for awareness of presence of stimulus in a shorter time window (up to 84 ms) than for awareness of stimulus features which require more detailed visual processing (up to 104 ms). This pattern of results is in line with those of Koivisto, Railo, and Salminen-Vaparanta (2011), who showed that the early visual cortex is necessary for a longer time window for discriminating more complex stimuli (such as an arrow shape) than for discriminating the orientation of a simple bar. Thus, the classical TMS-induced functional impairment taking place around 100 ms after TMS over the early visual areas (Amassian et al., 1989) may be composed of qualitatively different processes: the early part of it is related to awareness and perception of contrast change and basic orientation discrimination, whereas the later part is related to discrimination and awareness of more complex stimulus features.

Electrophysiological recordings in humans have shown that visual awareness is associated with brain activity at latencies later than the TMS-induced suppression in the present study, around 120–250 ms after stimulus onset (Koivisto & Revonsuo, 2010) or even later (Dehaene & Changeux, 2011). This suggests that the contribution of V2 is necessary at the early processing stages which precede and are prerequisites for the occurrence of visual awareness but which by themselves are not sufficient for visual awareness to emerge. Thus, assuming that visual awareness does not arise in V1 or V2, but somewhere in higher areas, our results imply that the higher areas cannot generate visual awareness without the stimulus being first processed both in V1 and V2.

5. Conclusion

The results showed that complete suppression of awareness of visual stimulus presence occurred when TMS pulse was delivered to V2 between 44 and 84 ms after visual stimulus onset, and suppression of awareness of stimulus features took place when the pulse was delivered between 44 and 104 ms after visual stimulus onset. These findings suggest that in addition to V1, whose role in visual awareness has been emphasised in the literature (e.g.

Pascual-Leone & Walsh, 2001; Tong, 2003), also V2 plays a critical role in visual awareness.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuropsychologia.2012.03.015.

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