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Waves of awareness for occipital and parietal phosphenes perception

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ABSTRACT

Transcranial magnetic stimulation (TMS) of the occipital cortex is known to induce visual sensations, i.e. phosphenes, which appear as flashes of light in the absence of an external stimulus. Recent studies have shown that TMS can produce phosphenes also when the intraparietal sulcus (IPS) is stimulated. The main question addressed in this paper is whether parietal phosphenes are generated directly by local mechanisms or emerge through indirect activation of other visual areas. Electroencephalographic (EEG) signals were recorded while stimulating left occipital or parietal cortices inducing phosphene perception in healthy participants and in a hemianopic patient who suffered from complete destruction of the early visual cortex of the left hemisphere. Results in healthy participants showed that the onset of phosphene perception induced by occipital TMS correlated with differential cortical activity in temporal sites while the onset of phosphene perception induced by parietal TMS correlated with differential cortical activity in the stimulated parietal site. Moreover, IPS-TMS of the lesioned hemisphere of the hemianopic patient with a complete lesion to V1 showed again that the onset of phosphene perception correlated with differential cortical activity in the stimulated parietal site. The present data seem thus to suggest that temporal and parietal cortices can serve as different local early gatekeepers of perceptual awareness and that activity in the occipital cortex, although being relevant for perception in general, is not part of the neural bases of the perceptual awareness of phosphenes.

1. Introduction

A central question in consciousness studies is to reveal which brain regions, and in what order of activation, critically determine specific conscious percepts. Several models have been proposed in order to find the brain areas ("where") correlating with visual awareness and to determine the time-course of neural activation in interconnected areas ("when/how") needed for awareness to emerge. With respect to the "where" question, one of the most influential models in visual processing, the so-called two-streams hypothesis (Goodale and Milner, 1992; Milner and Goodale, 2008), states that visual awareness is restricted to the ventral stream (Milner, 2012). The dorsal stream, instead, is thought not to be "in the business of providing any kind of a visual representation of the world" (Goodale and Milner, 2004, p. 114). With respect to the "when/how" question, another very influential model, Lamme's model (Lamme et al., 1998), states that recurrent processing feeding back to occipital cortex is necessary for awareness to emerge. On the basis of these two models, it can be predicted that the neural correlates of visual awareness can only be found along the ventral stream (comprising the occipital and temporal cortices) and that the integrity of the primary visual cortex (V1) is needed. Recent findings have challenged both of these statements. It has indeed been found that, at least under certain circumstances, the dorsal stream can generate visual awareness (Hesselmann and Malach, 2011; Koivisto et al., 2010; Mazzi et al., 2014) and that recurrent processing feeding back to V1 is not necessary for visual awareness (Zeki and ffytche, 1998; ffytche and Zeki, 2011; Mazzi et al., 2014).

In the present paper we further tested the contribution of the dorsal stream and V1 in the emergence of awareness by combining transcranial magnetic stimulation (TMS) and electroencephalography (EEG), two modern methodologies which can provide information both with respect to the "where" and the "when/how" questions.

TMS, by being a non-invasive direct stimulation method, is one

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of the state of the art methodologies used to study whether a specific neural area plays a crucial role in cognitive processes. The logic is the following: if TMS of a specific cortical area has a specific effect on performance, this area and the network associated to it are crucial for the studied function. In this respect, for example, if TMS is applied to the portion of the primary motor cortex (M1) representing the contralateral hand, a motor twitch of the hand contralateral to the stimulation is elicited, thus making it possible to conclude that activity within the stimulated area (M1) has a causal role in eliciting the motor twitch. Moreover, TMS can be useful not only for the localization of eloquent cortical areas but it can also determine the intensity needed to evoke a specific effect. For instance, by stimulating M1, one can determine the minimal TMS pulse intensity (the so-called motor threshold) needed to evoke a motor potential; this intensity is considered to directly reflect the level of excitability of the stimulated cortex.

Similarly, TMS of visual areas induces conscious visual percepts, or phosphenes, i.e. the experience of flashes of light in the absence of an external stimulus. Additionally, akin to the motor threshold, cortical excitability can be measured with the phosphene threshold. Typically, phosphene perception has been studied by stimulating areas within the occipital cortex. However, recent findings (Marzi et al., 2009; Mazzi et al., 2014; Fried et al., 2011) have shown that phosphenes can be elicited also along the dorsal stream (Goodale and Milner, 1992; Milner and Goodale, 2008), specifically in the intra-parietal sulcus (IPS). Interestingly for the purposes of the present paper, Mazzi and collaborators (2014) tested both healthy participants and hemianopic patients with a complete lesion of V1 and asked them to report the presence/absence of phosphenes while their occipital or parietal cortex was stimulated (only the parietal cortex was stimulated in hemianopic patients). The authors reported that, in healthy participants, IPS-phosphenes have a higher phosphene-threshold and different phenomenical characteristics than those elicited by occipital TMS. Importantly, they showed that parietal phosphenes can be obtained also in patients with a complete lesion to the ipsilateral V1 and that their conscious visual percepts were undistinguishable from those obtained with healthy participants. The authors concluded that (1) neural activity in V1 is not necessary for visual awareness and (2) that IPS is an independent generator of the awareness of phosphenes. As a note of caution, one should consider the possibility that the awareness of phosphenes generated by TMS of IPS could result from a spread of activity towards other visual areas (i.e. extrastriate areas and the temporal cortex). Although the involvement of V1 seems unlikely given the results with hemianopic patients, the awareness of IPS phosphenes could be induced by a "third visual area", or a network subtending connections with IPS, that could be in charge of providing access to visual awareness (Fried et al., 2011; Mazzi et al., 2014). A likely candidate for this hypothesis, as suggested by the previously described models (Goodale and Milner, 1992; Lamme et al., 1998) could be found along the ventral stream, e.g., in the temporal cortex which is known to play an important role in visual awareness (Goodale and Milner, 1992).

In order to test the contribution of such a "third area" in the generation of phosphenes, TMS-evoked potentials (TEPs) can be acquired by co-registering EEG signals while the cortex is stimulated. TEPs represent a clear and direct measure of cortical excitability and can be used to assess the state of cortical reactivity and connectivity also in the so-called silent-areas that do not produce a peripheral marker (Ilmoniemi et al., 1997). Here, we adopted an interactive approach (Miniussi and Thut, 2010) by using EEG–TMS co-registration while the participant performed a task. This approach consists in the stimulation of a circumscribed cortical area with TMS and to monitor, with EEG, the induced electrical changes in the whole cortex. EEG–TMS co-registration conceives the tracing of the time course of functionally relevant activity in distant but functionally connected areas relevant for the task at hand (i.e. effective connectivity). The relevance of this approach is twofold: (1) it provides an empirical measure of the network of areas implicated in a specific task as the activation induced by TMS of the targeted area propagates to functionally connected areas and (2) it provides information on the causal relationship in the connections across the network of activated areas. Given the high temporal resolution of EEG and the properties of the spreading of activity induced by TMS, if an area X results to be active prior to area Y it can be assumed that the activity in area X causes a change in the activity of area Y through effective connections between the two areas.

Thanks to these characteristics, TEPs are ideal to gather information on the time-course and spatio-temporal dynamics of the emergence of phosphene perception. Despite the relative crudeness of spatial topography of EEG, its temporal resolution is very high (in the range of milliseconds) and this is the most crucial characteristic for the purposes of the present paper. The logic is the following: if TEPs detect an electrical difference at the stimulated area (e.g. IPS) between phosphene-present and phosphene-absent trials in a specific time window after TMS, to be considered crucial for this effect, a "third area" (e.g. temporal cortex) should display a comparable effect (phosphene-present different than phosphene-absent) in the same or earlier time windows. If such a differential effect cannot be found, no causal role of the supposed "third area" (e.g. temporal cortex) can be advocated for the perception of phosphenes elicited by TMS of the stimulated area (e.g. IPS).

The main purpose of the present paper is to uncover the spatio-temporal dynamics of the onset of the phosphene perception as induced by occipital and parietal TMS. To do this, healthy participants and one hemianopic patient was asked to report the presence/absence of a phosphene induced by stimulation of a specific occipital and parietal site by means of TMS while monitoring and recording TEPs. On the basis of the results of this EEG–TMS interactive co-registration approach, we could draw some conclusions both on the role of V1 in perceptual awareness and on whether or not other functionally interconnected areas are playing some role in the emergence of awareness after stimulation of the occipital and parietal cortex.

2. Experiment 1

2.1. Materials and methods

2.1.1. Healthy participants

Sixteen healthy volunteers (9 females) were recruited to participate in the study. Their ages ranged between 22 and 28 years (mean 25 years, sd 1.90) and they were all right handed, as assessed with the Edinburgh Handedness Inventory (Oldfield, 1971). They all had normal or corrected-to-normal visual acuity and no history of neurological or psychiatric disorders. All participants gave their written informed consent prior to participation. The experiment was carried out according to the principles laid down in the 1964 Declaration of Helsinki and approved by the local Ethics Committee. As assessed by a safety screening questionnaire (adapted from Rossi et al., 2011), participants were negative for all risk factors associated with TMS: none reported neurological disorders, cardiac pacemaker, any history of epilepsy or migraine, current treatment with any psychoactive medication and pregnancy. One participant could not perceive reliable phosphenes after either occipital or parietal sites and four participants dropped out and did not perform the second session. These participants were thus excluded from the sample.

2.1.2. Experimental procedure

Participants were individually tested in a dimly illuminated room. During the experiment, they sat in front of a 17 in. LCD monitor (LG L1753HM) at a viewing distance of 57 cm with their head secured in a chin rest with forehead support. To enhance the excitability of their visual cortex, participants' eyes were covered with eye-patches prior to the threshold measurements and they performed the entire experiment blindfolded and were asked to maintain their gaze steady in front of them. Commercial earplugs were used with the aim of protecting the participants from the noise associated with TMS (Rossi et al., 2009) and preventing responses from being affected by the intensity of the coil click.

At the beginning of each session, after applying the cap with electrodes for EEG recording, phosphene threshold (PT) was assessed for occipital and parietal sites, by means of an automatic, non-adaptive, psychophysical method ("Method of constant stimuli") implemented with Matlab (Abrahamyan et al., 2011). Thirteen randomly intermixed different intensities were employed (ranging from 57% to 93% of maximum stimulator output (MSO), with changes in steps of 3%) and eight pulses were given for each stimulator output intensity (total number of pulses = 104). The data obtained were then fitted with a cumulative Weibull psychometric function via a maximum likelihood criterion using the Palamedes toolbox (http://www.palamedestoolbox.org) with Matlab. The stimulation intensity at which the participant could perceive a phosphene on 50% of trials was taken as the threshold value and used in the subsequent experimental session.

The experimental session comprised 4 blocks of 80 trials each, for a total number of 320 stimulations. After each TMS pulse, participants were requested to report the presence or absence of a phosphene with a "yes/no" response by pressing respectively the "z" button (left index finger) or the "m" button (right index finger) on the keyboard without losing time. After the response was given, there was a random interval (ranging from 3000 to 3300 ms) and then the subsequent pulse was automatically delivered. The inter-pulse interval was never shorter than 4 s, well above the criterion assessed by safety instructions (Anand and Hotson, 2002; Wassermann, 1998).

Each participant performed two experimental sessions (conducted on separate days with at least 48 h between sessions), one for each stimulation site (left occipital cortex and left parietal cortex). The order of the two sessions was counterbalanced among participants. In total, each experimental session lasted about two and a half hours, including the setup of the EEG cap and neuronavigation system. Participants were debriefed at the end of the second experimental session.

2.1.3. TMS protocol

For each experimental session, single-pulse magnetic stimulation (inter-pulse interval >4 s) was delivered through a 70 mm figure-of-eight coil connected to a biphasic Magstim Rapid^2 system (maximum output 3.5 T) (Magstim Company Limited, Whitland, UK). The TMS pulse trigger and response acquisition were controlled with Matlab (The MathWorks, Natick, MA) for the phosphene threshold assessment, and with E-Prime 1.1 (SP3) software (Psychology Software Tools, Pittsburgh, PA) for the experimental session.

Neuronavigation software (SofTaxic, E.M.S., Bologna, Italy) combined with a 3D optical digitizer (Polaris Vicra, NDI, Waterloo, Canada) was used throughout the experiment to maintain the coil position over the participant's head within a 2 mm accuracy threshold. The TMS coil was placed tangentially on the surface of the scalp, parallel to the participant's sagittal midline, with the handle pointing upwards in order to avoid unspecific activation of neck and shoulder muscles.

The best location found for eliciting circumscribed and right-lateralized phosphenes, the "hot spot", was then acquired by the neuro-navigation system and the coil was fixed in the targeted position by means of a mechanical arm (Manfrotto magic arm, Italy, www.manfrotto.com). To confirm that participants perceived genuine phosphenes, some criteria (Kammer et al., 2005) such as the dependence on the stimulated hemisphere, i.e. phosphenes in the contralateral visual field (Meyer et al., 1991), gaze direction (Meyer et al., 1991)and visibility with the eyes both open and closed (Kammer and Beck, 2002), had to be satisfied during training trials. In order to avoid any potential effects of participants' trying to comply with the experimenter's expectations, additional tests were performed (see Mazzi et al., 2014 for details).

Since previous studies reported that left hemisphere stimulation evokes more reliable phosphenes than the right hemisphere (Beckers and Hömberg, 1992; Stewart et al., 1999; Antal et al., 2001; Silvanto et al., 2008), we applied TMS over the left hemisphere. Individual hot spots were located using the functional method of inducing phosphenes by stimulating with suprathreshold intensities in a region within an area of 2 cm in diameter centered on two different scalp positions (see Fig. 1A): (1) the occipital lobe in correspondence to the O1 electrode position of the 10–20 International EEG system and (2) the parietal lobe in correspondence to the P3 electrode position of the 10–20 International EEG system. These sites are most likely to correspond, respectively, to visual cortical areas V1/V2 (Thielscher et al., 2010, Salminen-Vaparanta et al., 2012) and intraparietal sulcus in all participants (Mazzi et al., 2014).

2.1.4. EEG recording and TMS-evoked potentials (TEPs) analysis

TMS-compatible EEG equipment (BrainAmp, Brain Products GmbH, Munich, Germany) was used to record EEG signals (BrainVision Recorder). The EEG activity was continuously recorded from a Fast'n Easy cap with 27 TMS-compatible Ag/AgCl pellet pin electrodes (EasyCap GmbH, Herrsching, Germany) placed according to the 10–20 International System (O1, O2, P7, P3, Pz, P4, P8, CP5, CP1, CP2, CP6, T7, C3, Cz, C4, T8, FC5, FC1, FC2, FC6, F7, F3, Fz, F4, F8, Fp1, Fp2). Additional electrodes were used as reference and ground and for the electro-oculogram. The ground electrode was placed in AFz, i.e. at the maximal distance from the stimulating TMS coil. All scalp channels were online referenced to the right mastoid (RM) and then re-referenced offline to the left mastoid (LM). Horizontal and vertical eye movements were detected respectively with electrodes placed at the left and right canthi and up and below the right eye. The impedance of all the electrodes was kept below 5 kΩ. The EEG was recorded at 5000 Hz sampling rate with a time constant of 10 s as low cut-off and a high cut-off of 1000 Hz. The EEG signal was processed off-line using BrainVision Analyzer 1.05.

In order to reduce TMS artifacts and to make possible to record the EEG signals from the electrodes placed right beneath the TMS coil, we devised a custom-made polystyrene C-shaped annulus. The annulus was positioned over the stimulated electrodes (O1, P3), making it possible to place the coil over the target electrode without the need to physically remove that electrode.

Continuous data were filtered offline with a 20 Hz (12 dB/octave) (Oruç et al., 2011) high cut-off filter and then divided into epochs starting from 200 ms before and ending 600 ms after the TMS pulse. Epochs were then baseline corrected (from -200 ms to 0 ms) and visually inspected in order to remove all trials contaminated by eye movements and blinking artifacts, involuntary motor acts or excessive noisy EEG. After pre-processing, the average number of trials were 102.22 for "yes" responses and 74.11 for "no" responses when stimulating the occipital cortex and 90.67 "yes" responses and 112.33 "no" responses when stimulating the parietal cortex. TEPs were obtained by averaging epochs for each participant and for each stimulation site separately for trials where

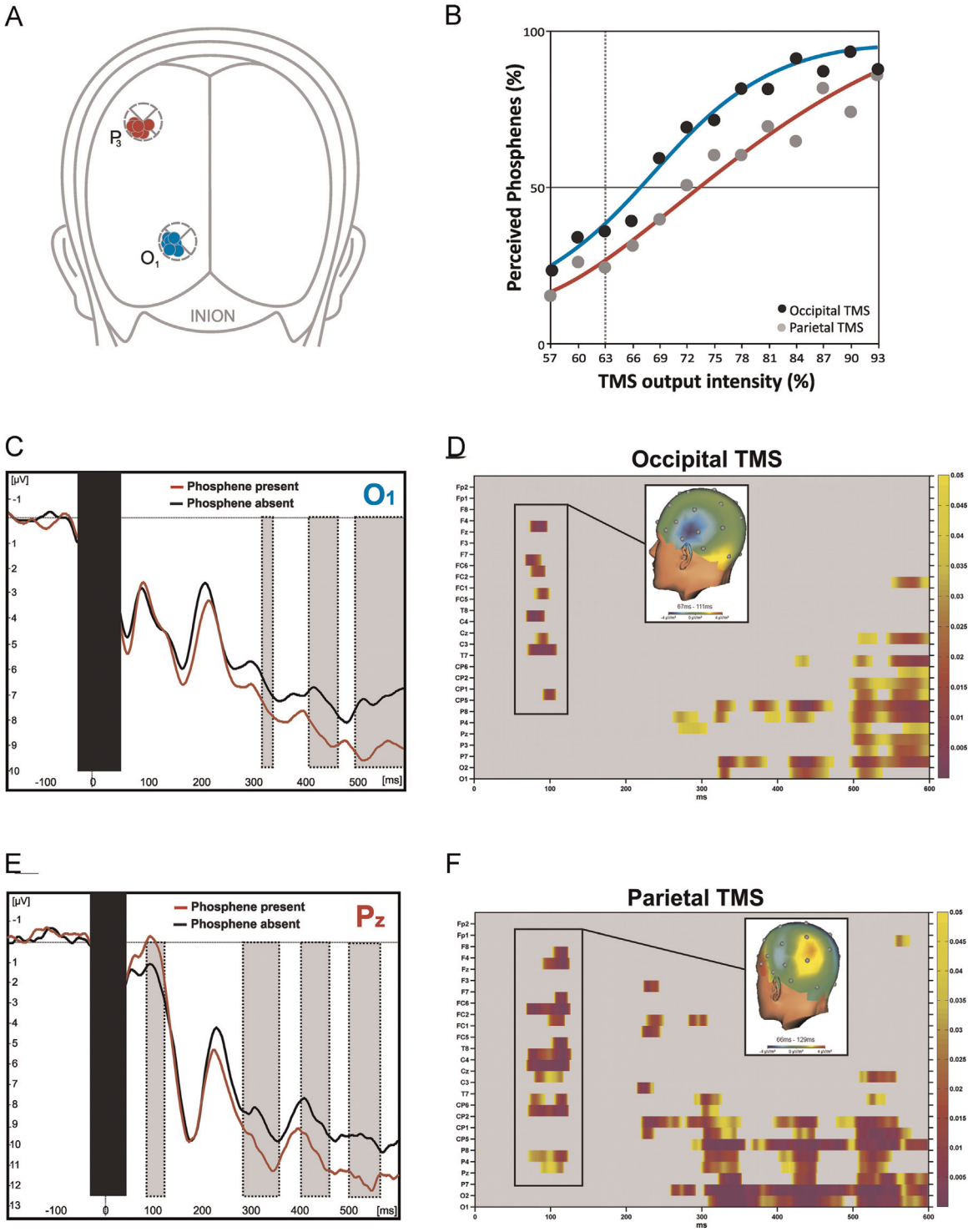

Fig. 1. Experiment 1. Results and analyses with healthy participants. (A) Individual hot-spots for occipital (blue) and parietal (red) stimulation sites eliciting reliable phosphenes. (B) Psychometric functions for the two sites of stimulation. The blue line indicates the threshold function obtained after TMS of the occipital cortex while the red line represents the threshold function obtained after TMS of the parietal cortex. Each dot indicates the mean performance across subjects at each TMS intensity for the two stimulation sites. (C) TEPs elicited by TMS pulses over O1 as a function of the phosphene-present (red) and phosphene-absent (black) conditions. Gray dotted boxes show the time windows in which the two waveforms are statistically significant. The black box indicates the time window in which the TMS artifact was present. (D) Intensity plots showing the cluster-corrected significant time windows resulting from the two-tailed point-wise paired *t*-tests comparing phosphene-present and phosphene-absent condition following O1-TMS. The *x*-, *y*- and *z*-axis represent, respectively, time (from 0 ms to 600 ms after TMS pulse), electrodes and *t*-test values (depicted by different colors) at each data point. The figure inset depicts the SCD topographic map of the phosphene present/absent effect. The SCD focus is compatible with temporal generators. (E) TEPs elicited by TMS pulses over P3 as a function of the phosphene-present (red) and phosphene-absent (black) conditions. Gray dotted boxes show the time windows in which the two waveforms are statistically significant. The black box indicates the time window in which the TMS artifact was present. (F) Intensity plots showing the cluster-corrected significant time windows resulting from the two-tailed point-wise paired *t*-tests comparing phosphene-present and phosphene-absent condition following P3-TMS. The *x*-, *y*- and *z*-axis represent, respectively, time (from 0 ms to 600 ms after TMS pulse), electrodes and *t*-test values (depicted by different colors) at each data point. The figure inset depicts the SCD topographic map of the phosphene present/absent effect. The SCD focus is compatible with parietal generators. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

participants reported perceiving a phosphene (hereafter called "phosphene-present" trials/conditions) and those where TMS did not elicit any visual percepts (hereafter called "phosphene-absent" trials/conditions).

Given that TMS artifacts influence the recording of meaningful EEG data in a period of a few milliseconds after delivery of the magnetic pulse (Veniero et al., 2009), data recorded up to 50 ms after the TMS pulse was not analyzed. Due to the presence of noisy EEG signals in two participants, TEP analysis was performed on the data from 9 participants (7 females; mean age 24.78 years, sd 1.79 years). Moreover, despite the presence of the custom-made annulus over the stimulated electrode, because of the arrangement of the electrode's lead wire (Sekiguhi et al., 2011) strong TMS artifacts were recorded from the P3 electrode in some participants when the parietal cortex was stimulated. We therefore analyzed TEPs recorded from Pz in all participants, which was the electrode closest to the stimulation site with EEG signals free of TMS artifacts. Instead, TMS of the occipital cortex did not induce such strong artifacts and the data obtained from the O1 electrode could be analyzed.

2.1.5. Statistical analysis

Behavioral and electrophysiological analysis were performed on nine participants. For the statistical analysis of the phosphene threshold functions obtained for the two stimulated sites, a one-tailed Kolmogorov–Smirnov test was applied. Response times for the phosphene-present and phosphene-absent trials were analyzed using a 2-way repeated measures ANOVA. TEPs amplitude was analyzed separately for each stimulation site using two-tailed point-wise paired *t*-tests (Guthrie and Buchwald, 1991) to compare trials where a phosphene was perceived (phosphene-present) and trials where TMS pulse did not evoke any visual percepts (phosphene-absent). The 0.05 α criterion was set as the significance level. The minimum number of consecutive significant data points (cluster) needed to control for the family-wise error rate was set at 101 (>20 ms at a 5000 Hz digitizing rate). The results of this analysis are represented as intensity plots (Murray et al., 2002) in which only cluster-corrected significant results are shown (see Fig. 1D and F) to depict the topographic distribution of differential activation associated with phosphene-present trials versus phosphene-absent trials and to identify the onset of these differences across time.

Scalp current density (SCD) maps were used to determine the generators contributing to the phosphene present/absent effect. SCD maps are based on the Laplacian second derivative of the field potential and have the advantage to be directly proportional to the current density, to be reference-independent and to mathematically eliminate the voltage gradients due to tangential current flows. Thus SCD maps emphasize the local contributions to the surface maps and provide a better localization of approximate locations of intracranial generators. In the present experiment SCD topographic maps were computed from the spherical spline interpolation of the surface voltage recording (Perrin et al., 1989), obtained for the phosphene present/absent effect, as implemented in BrainVision Analyzer 2.0. A fourth-order spherical spline was used with a spline-smoothing coefficient (λ) of 1×10^{-6}. In order to obtain the highest signal-to-noise ratio and to account for inter-individual differences, SCD maps were created on the group-averaged TEP data. Given that the previous point-wise paired *t*-tests showed a broadly distributed effect in late time windows, we limited our SCD analysis to the early time window, i.e. the one signalling the onset of the phosphene present/absent effect. Moreover, in order not to bias the SCD topographic maps, we created a single map for the entire early time-window where the phosphene present/absent effect resulted in being significant in at least one electrode (i.e. from 67 to 111 ms for occipital TMS and from 66 to 139 ms for parietal TMS). To determine the display gain of the maps we visually inspected the SCD maps in the baseline period (from -200 ms to 0 ms) in order to appreciate the contribution of noise to the SCD topographic maps.

2.2. Results and discussion

2.2.1. Phosphene threshold function

Each participant was able to perceive reliable phosphenes induced by stimulation to the left occipital and left parietal cortices (see Fig. 1A for individual stimulation sites). Induced phosphenes from stimulation of both target sites consisted of brief static flashes of light, mostly greyish or white, appearing in the hemifield contralateral to TMS. Individual occipital phosphene thresholds ranged between 60% and 76% of the MSO (mean=66.89, sd=4.54), while parietal thresholds ranged between 60% and 81% of the MSO (mean=72.56, sd=7.5). One-tailed Kolmogorov–Smirnov test showed that the mean threshold functions obtained for the two stimulated sites were different starting from 63% MSO ($X^2=0.084$, $p<0.05$) (see Fig. 1B). This result, in line with that obtained by Fried et al. (2011) and Mazzi et al. (2014), indicates that the level of stimulation necessary to generate conscious visual percepts after TMS of the occipital and parietal cortex starts to be different at a low intensity and remains different for all the upper parts of the threshold function.

2.2.2. Behavioral results

With respect to the occipital cortex, phosphenes were evoked on average in 59.81% of the trials (sd=9.50), while the remaining 40.19% of trials resulted in no phosphene perception. TMS of the parietal cortex induced phosphene perception in 42.29% of the trials (sd=12.29), while the remaining 57.71% of trials resulted in no phosphene perception, indicating that the percentage of phosphene detections was roughly 50% in both stimulated sites. Moreover, for both the occipital and parietal TMS, the TMS pulse intensity remained the same during the entire experimental session, allowing to assume that the trials were only different for the subjective report: i.e. the presence or absence of a phosphene.

A 2-way repeated measures ANOVA on response times with Stimulation Site (O1/ P3) and Phosphene Perception (present/absent) as within-subject factors was carried out. Results showed a significant main effect of Phosphene Perception [$F_{(1,8)}=8.81$, $p<0.05$, $\eta_p{}^2=0.52$] indicating that phosphene-present trials (958.12 ms) were reacted to faster than phosphene-absent trials (1128.98 ms), thus providing evidence for the reliability of participants' self-reports (i.e. the presence/absence of a visual percept). Furthermore, the results showed a tendency toward significance for Stimulation Site [$F_{(1,8)}=4.69$, $p=0.06$, $\eta_p{}^2=0.37$], indicating faster responses when TMS was applied over the parietal cortex (986.43 ms) compared to occipital stimulation (1100.70 ms). These results are in line with those obtained by Marzi et al. (2009) who reported shorter reaction times for phosphenes obtained by parietal stimulation compared to occipital phosphenes. Finally, the interaction was found not to be significant [$F_{(1,8)}=3.31$, $p=0.11$, $\eta_p{}^2=0.29$], indicating that the speeding up of response times induced by phosphene perception was not significantly different between the two stimulated sites.

2.2.3. TEPs results

To investigate how TEPs amplitude was modulated by the perception of a phosphene and to better characterize the spatio-temporal dynamics of the brain's responses to the presence/absence of the phosphene induced by TMS, we entered TEPs amplitude recorded at 27 of the 32 electrode locations (i.e. excluding the left mastoid and the electrodes used to record vertical and horizontal eye movements) into point-wise paired *t*-tests for TMS

of the occipital (Fig. 1C and D) and parietal cortices (Fig. 1E and F). Cluster plots (Fig. 1D and F) represent the topographic distribution over time for the phosphene present/absent effect (i.e. significant differences between phosphene-present and phosphene-absent conditions are shown). Moreover, SCD topographic maps (insets of Figs. 1D and F) display the generators of the effect for the early time window. Analyses were performed separately for the two sites of stimulation. Given that a difference in SCD topographic maps implies different configurations of generators in the brain (Vaughan, 1982; Michel et al., 2004), if the SCD maps of two stimulation sites are different, we can conclude that the two stimulation sites are different with respect to the topography of their effects.

2.2.3.1. Occipital cortex. When single-pulse TMS was applied over the left occipital cortex, phosphene-present and phosphene-absent TEPs amplitude recorded at the O1 electrode (Fig. 1C) differed significantly in three late time windows: between 319.2 ms and 340.3 ms (lasting 21.1 ms), between 413.4 ms and 470.4 ms (lasting 57 ms) and between 501.6 ms and 600 ms (lasting 98.4 ms) after the TMS pulse. In all the time windows, phosphene-present trials were characterized by a higher amplitude than the phosphene-absent trials.

The earliest onset latency of the phosphene present/absent effect (i.e. a significant difference in TEPs amplitude for phosphene-present versus phosphene-absent trials) in the stimulated hemisphere is found at 67.2 ms after the TMS pulse at scalp site T7 (Fig. 1D). In the same early time window (onset latencies up to 90 ms) other central scalp sites showed a significant effect in both hemispheres (C3, CP5, FC5, Fz, C4, FC2, FC6). Importantly, the SCD topographic map at the early time window showed that the phosphene present/absent effect occurs over temporal areas. Significant effect at posterior sites are only evident at later onset latencies, starting at ~260 ms after the TMS pulse at parietal sites (Pz, P4), then extending to centro-parietal (CP5) and occipital (O1, O2) sites at ~320 ms after TMS pulse with massive significant effects at almost all posterior sites starting at ~500 ms after TMS pulse.

2.2.3.2. Parietal cortex. When single-pulse TMS was applied over the left parietal cortex, phosphene-present and phosphene-absent TEPs amplitude recorded at Pz electrode (Fig. 1E) were reliably different in the same three late time windows found for occipital TMS: between 283.6 ms and 352.4 ms (lasting 68.8 ms), between 402 ms and 455 ms (lasting 53 ms) and between 504.6 ms and 550.8 ms (lasting 46.2 ms) after the TMS pulse. In contrast to occipital TMS, parietal TEPs were additionally different in one early time window between 84.2 ms and 119.4 ms after TMS (lasting 35.2 ms). Phosphene-present trials for parietal stimulation were characterized by a higher amplitude than phosphene-absent trials in all the time windows, similar to results found in occipital stimulation

The earliest onset latency of the phosphene-present/absent effect in the stimulated hemisphere was found at 66.2 ms after the TMS pulse at scalp site Cz (Fig. 1F). In the same early time window (onset latencies up to 100 ms) other central scalp sites showed a significant effect in both hemispheres (C3, C4, Pz, P4, CP2, CP6, T8, FC1, Fz, F4, FC2). The SCD topographic map at the early time window showed that the phosphene present/absent effect occurs over centro-parietal areas (see Fig. 1F inset). A second phase of the phosphene present/absent effect started at ~210 ms after TMS in several left hemisphere sites (CP1, CP5, T7, FC1, FC5, F7) while a massive significant effect at posterior sites (O1, O2, P7, Pz, P4, P8, CP1, CP2) was only evident at later onset latencies starting at ~280 ms after the TMS pulse and persisting up to the end of the analyzed window with a ~10 Hz onset frequency.

Taken together, the data of the present experiment showed that the time-course of phosphene perception following occipital and parietal TMS is different, with an earlier onset for parietal than occipital phosphenes as recorded at the stimulation sites. Analysis of the scalp distribution (Fig. 1D and F) of the phosphene present/absent effect also revealed that the generation of a phosphene after TMS of the two stimulated sites (occipital vs. parietal) correlated with activity at occipital sites in the late phase, whereas a different localization can be seen for the early phase: occipital phosphenes correlated with the activity of centro-temporal left sites (T7, C3, CP5, FC5) while parietal phosphenes correlated with the activity of central, parietal and frontal sites (C3, Cz, Pz, FC1, Fz). Importantly, SCD topographic maps at the early phase are consistent with temporal generators for occipital phosphenes and parietal generators for parietal phosphenes (Fig. 1D and F insets).

3. Experiment 2

In the present experiment we further tested the independence of the parietal cortex in phosphene perception in a patient with a complete lesion of the left primary visual cortex. If neural activity in V1 is necessary for the perception of parietal phosphenes, we should find no evidence of the presence of phosphenes after parietal TMS. Alternatively, if the patient would show the same behavioral performance of the healthy participants in Experiment 1 and, more importantly, the same time-course of the TEPs, we could conclude that feedback to V1 is not necessary for awareness to emerge. Moreover, a spatio-temporal analysis of the time-course of the phosphene present/absent effect will also serve to establish the possible contribution of other areas in the generation of a phosphene after TMS to the parietal cortex. In this respect, if no other areas (i.e. temporal cortex) respond differently to the onset of the phosphene present/absent effect, we could conclude that the parietal cortex is an early independent generator of awareness if directly stimulated with TMS.

3.1. Materials and methods

3.1.1 Patient SL

The hemianopic patient S.L. (right handed female, 44 years old) suffered a right homonymous hemianopia (Fig. 2B) resulting from an ischemic stroke with hemorrhagic evolution. MRI evidenced a complete destruction of the left striate cortex (V1) (Fig. 2A). The absence of islands of residual functionality in the left V1 of patient SL was tested in a previous paper (Mazzi et al., 2014): briefly, TMS was applied at a supra-threshold intensity in different portions of the lesioned occipital cortex. A total of 25 sites (separated by 1 cm) were stimulated, with each site being stimulated five times, for a total of 125 TMS pulses. This procedure did not elicit any conscious visual percepts, thus demonstrating no residual functionally active visual areas within the lesion.

Visual field defect was assessed by means of a computerized perimetry (Humphrey system). The patient was tested in 2013, about 50 months after her neurological event. The patient provided her written informed consent prior to participating in the study and she was free to withdraw at any time. The experiment was carried out in accordance with the 1964 Declaration of Helsinki and approved by the local Ethics Committee.

3.1.2. TMS protocol, EEG recording and experimental procedure

The TMS protocol, EEG recording and experimental procedure were identical to that described in Experiment 1 with healthy participants, with the exception that TMS was applied only to the parietal cortex of the left damaged hemisphere (corresponding to the P3 electrode). The same criteria used for assessing the

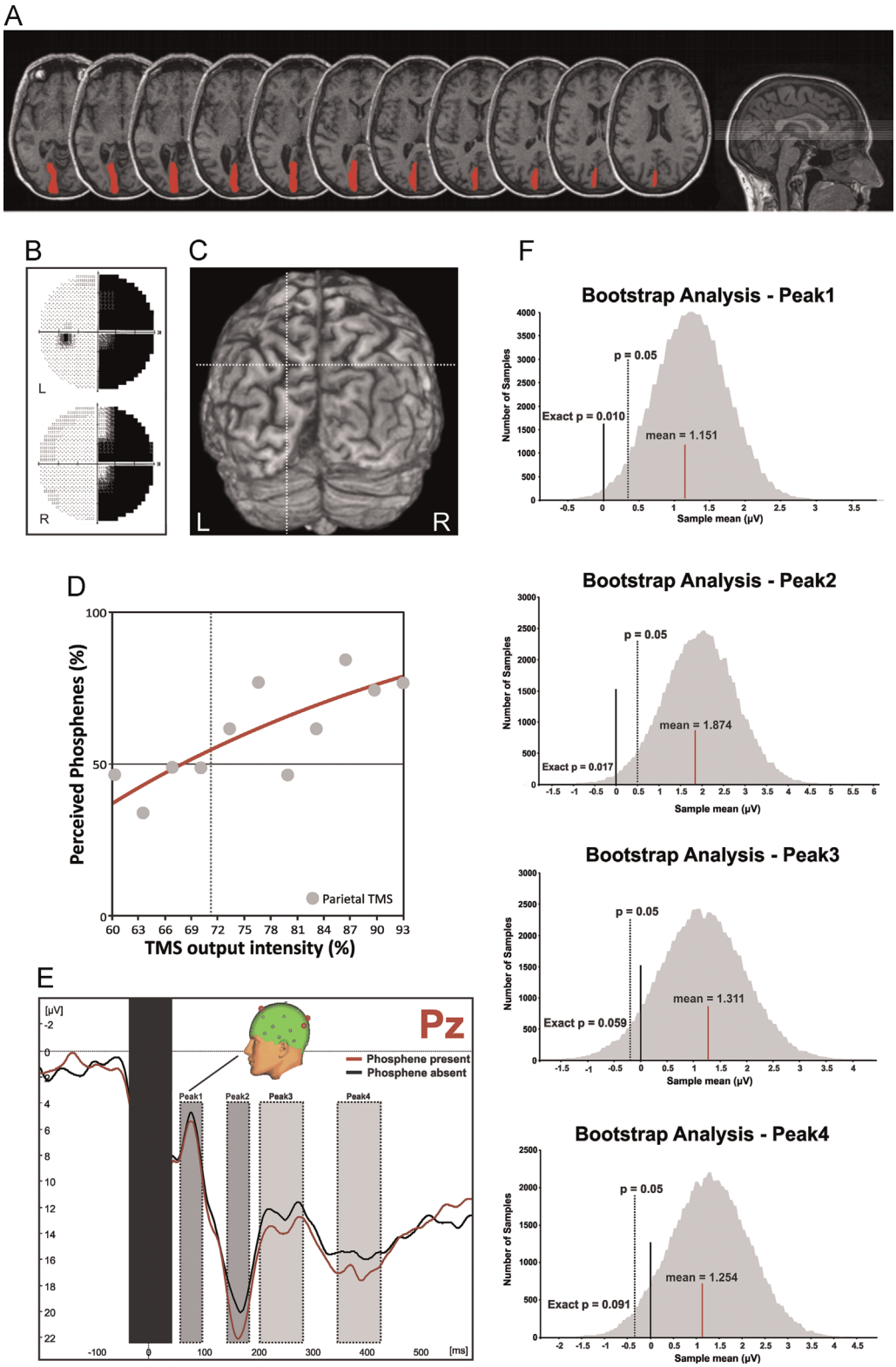

Fig. 2. Experiment 2. Results and analyses with hemianopic patient SL. (A) Brain lesion reconstruction. (B) Visual field defect. (C) Site of stimulation. The intersection of the dashed lines, superimposed on the 3D reconstruction of the patients' brain, represents the hot-spot for parietal stimulation. (D) Psychometric threshold function obtained with patient SL. The red line represents the threshold function obtained after TMS of the parietal cortex. Each dot indicates the patient's performance at each TMS intensity. (E) TEPs elicited by TMS pulses over P3 as a function of the phosphene-present (red) and phosphene-absent (black) conditions. Dotted boxes mark the time windows in which statistical analysis were performed. Dark gray boxes indicate statistically significant results while light gray boxes the time windows resulted to be not significant. The black box indicates the time window in which the TMS artifact was present. The figure inset depicts the left hemisphere electrodes (red circles) significant at the early time window. (F) Illustration of the results of the bootstrap analysis performed at each of the four waveform peaks. Solid red bars indicate the mean amplitude of the difference between phosphene-present and phosphene-absent trials. Solid black lines correspond to zero. Dotted black lines mark the 5th percentile value which, if above zero, indicate a statistically significant difference (Peaks 1 and 2). The exact *p*-values correspond to the proportion of resamples that were smaller than zero. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

genuineness of phosphenes in the healthy group was used for patient SL.

3.1.3. Behavioral data statistical analysis

To compare the performance of patient SL with that of the healthy participants we used two tests developed by Crawford and colleagues which are considered the most suitable analyses when the normative sample is small. Specifically, we used (1) the Singlims_ES.exe program (Crawford and Garthwaite, 2002; Crawford and Howell, 1998) which tested the hypothesis that the score of a single individual lies within or outside the normal range of scores defined by the mean and standard deviation of control group's data and (2) the RSDT_ES.exe program (Crawford and Garthwaite, 2005; Crawford et al., 2010) to compare differences between single-case scores on two conditions and the differences observed in a control sample. See Mazzi et al. (2014) for a more detailed description of the tests.

3.1.4. TEPs analysis

For homogeneity with the analysis performed with healthy participants we analyzed TEPs recorded at the Pz electrode (in the present case EEG recorded at P3 electrode was not affected by TMS artifacts, data from the P3 electrode is thus reported in the subsequent spatio-temporal analysis). By visually inspecting the averaged TEPs as recorded at the Pz electrode, four time windows with amplitude peaks could be identified: around ~80 ms (phosphene present: 78 ms; phosphene absent: 77 ms), ~170 ms (phosphene present: 166 ms; phosphene absent: 171 ms), ~250 ms (phosphene present: 250 ms; phosphene absent: 253 ms) and ~395 ms (phosphene present: 393 ms; phosphene absent: 402 ms) after the TMS pulse (Fig. 2E). Peak latencies were detected separately for the two conditions and the different time windows by means of the peak latency detection module implemented in BrainVision Analyzer 1.05. Given that using a single peak value when analyzing single trials data can be very noisy (Oruç et al., 2011), we used the peak export solution implemented in BrainVision Analyzer 1.05 to export, for each condition and time window, the mean amplitude values in a time window centered on the previously identified peaks at an average level (a 40-ms time window for the first two peaks and 80-ms for the two later peaks). After pre-processing the EEG data, the phosphene-present and phosphene-absent conditions resulted in 164 and 177 trials, respectively. Given that we cannot predict which trial in the sequence would result in a phosphene-present or absent outcome and, more importantly, that the two conditions did not result in the same number of trials, before running the analysis, the order of trials was randomized within each of the two conditions (phosphene present/absent). For single-subject analysis we used the first 164 trials per condition and we calculated the difference between the mean amplitude for phosphene-present and phosphene-absent trials per each trial pair. The difference in amplitude for the two conditions was then analyzed by means of a non-parametric Monte Carlo percentile bootstrap simulation (Efron and Tibshirani, 1993; Oruç et al., 2011). This procedure creates a simulated data distribution by re-sampling the raw data with replacement. We created 50,000 re-samples of 164 trials each for the phosphene-present minus phosphene-absent amplitude values. The lower 5^{th} percentile of the re-sampled data distribution served as the critical values for the one-tailed 0.05 significance level. If the 5th percentile results to be above the zero level (phosphene-present > phosphene-absent), it means that the phosphene-present condition yields a significantly larger amplitude than the phosphene-absent condition. This analysis was performed separately for each of the four time windows. For the spatio-temporal analysis involving the other electrodes we adopted the same procedure described above. In a visual inspection of the entire set of electrodes, three peaks can be detected (the last window peaking at ~395 ms after TMS was visible only for Pz). The first peak detectable by visually inspecting the TEPs had a latency of ~80 ms for electrodes O1, O2, P7, P3, Pz, P4, CP5, CP1, T7, C3, Cz, FC5 and F7, of ~95 ms for electrodes CP2, C4, FC1, FC2, F3 and F4, and of ~110 ms for electrodes P8, T8, CP6, FC6, F4 and Fz. The second peak, with a latency of ~165/170 ms after TMS was visible for all electrodes (O1, O2, P7, P3, P4, P8, CP5, CP1, CP2, CP6, T7, C3, Cz, C4, T8, FC5, FC1, FC2, FC6, F7, F3, Fz, F4, F8). The third peak was detectable at ~250 ms after TMS for electrodes P3 and P4 and at ~280 ms after TMS for electrode P8. No later peaks could be identified for any electrodes with the exception of Pz (see above) and no analysis was performed for this time window. Electrodes Fp1 and Fp2 did not show any clear peaks at any time windows and were not further analyzed.

3.2. Results and discussion

3.2.1. Phosphene threshold function

SL, as previously reported (Mazzi et al., 2014), could experience phosphenes when TMS was applied over the parietal cortex of her lesioned hemisphere (Fig. 2C) with eyes both open and blindfolded. In the previous paper and in the present one, induced phosphenes were localized in the upper right hemifield, contralateral to the stimulation site, and were described as very brief, static and mostly greyish or white.

The maximum stimulator output (MSO) intensity capable of eliciting a phosphene in 50% of trials was 71%, a value not significantly different [$t_{(8)}=-0.197$; $p=0.85$, Z-CC$=-0.208$] from the mean threshold values obtained stimulating the left parietal cortex of healthy participants in Experiment 1. These results are in line with those obtained by Mazzi et al. (2014) reporting that a psychophysical threshold function (Fig. 2D) for parietal phosphene perception could be created also for a patient lacking V1 and that her threshold function did not differ from the function obtained with healthy participants.

3.2.2. Behavioral results

When stimulating the ipsilesional parietal cortex (P3), SL reported perceiving a phosphene in 47.22% of the trials ($N=170$), while single-pulse TMS did not elicit a phosphene in the remaining 52.78% of trials ($N=190$). The percentage of perceived parietal phosphenes did not differ from the mean percentage value obtained in Experiment 1 with healthy participants [$t_{(8)}=0.381$; $p=0.71$, Z-CC$=0.401$]. Moreover, the patient's response times showed a speeding up in phosphene-present (mean$=1459.07$ ms) versus phosphene-absent (mean$=1702.79$ ms) trials that was comparable [$t_{(8)}=0.303$; $p=0.77$, Z-DCC$=0.356$] to that observed during parietal stimulation of healthy participants in Experiment 1, thus providing evidence of the reliability of patient's self-reports (i.e. the presence/absence of a visual percept).

3.2.3. TEPs results

Fig. 2E and F shows TEPs recorded at electrode Pz for left parietal cortex stimulation and the histograms of the phosphene-present/phosphene-absent contrast values obtained from the bootstrap analysis separated for the four time windows. As it can be seen from the figures, the first two peaks were significant (Peak 1, Exact $p=0.010$; Peak 2, Exact $p=0.017$), both having more positive power values for the phosphene-present condition. The last two peaks (at ~250 and ~395 ms after TMS pulse) were instead not significant (Peak 3, Exact $p=0.059$; Peak 4, Exact $p=0.091$). Importantly, the first peak (at ~80 ms after TMS pulse) indexes the early onset of the phosphene present/absent effect as detected at the Pz electrode.

The spatio-temporal analysis revealed that, in addition to Pz, a significant early difference between phosphene-present and

phosphene-absent responses was found at ~79 ms after the TMS pulse at scalp site P3 (phosphene-present peak: 80 ms; phosphene-absent peak: 78 ms, Exact $p=0.027$) and at ~104 ms after the TMS pulse at scalp site Fz (phosphene-present peak: 105 ms; phosphene-absent peak: 103 ms, Exact $p=0.013$). At the second peak, in addition to Pz, a significant difference was found at scalp sites P3 (phosphene-present peak: 163 ms; phosphene-absent peak: 169 ms, Exact $p=0.0003$), P4 (phosphene-present peak: 168 ms; phosphene-absent peak: 170 ms, Exact $p=0.0069$), P8 (phosphene-present peak: 170 ms; phosphene-absent peak: 172 ms, Exact $p=0.0092$), CP1 (phosphene-present peak: 162 ms; phosphene-absent peak: 164 ms, Exact $p=0.0088$), CP2 (phosphene-present peak: 164 ms; phosphene-absent peak: 168 ms, Exact $p=0.015$), CP6 (phosphene-present peak: 168 ms; phosphene-absent peak: 172 ms, Exact $p=0.047$). At the third peak none of the analyzed electrodes (P3, P4 and P8) showed a significant difference between phosphene-present and phosphene-absent responses (Exact $p > 0.05$).

Taken together, these results, in line with those obtained with healthy participants, show that the onset of the phosphene present/absent effect can be found at parietal sites (P3, Pz) and that the effect spreads to circumscribed functionally connected centro-parietal sites. Given that temporal sites do not show any effect, it can be concluded that these sites are not part of the functional network generating the effect, thus reinforcing the idea that the parietal cortex is an early and independent generator of phosphenes. Moreover, the presence of this effect in a patient with a complete lesion to the ipsilateral occipital cortex is in line with the idea that feedback to V1 is not necessary for the awareness of parietal phosphenes.

4. General discussion

The present paper aimed to establish the neural dynamics of the awareness of occipital and parietal phosphenes by investigating TEPs in healthy participants and one hemianopic patient with a lesion to V1. Phosphenes were induced by stimulating V1/V2 and IPS while EEG signals were recorded. In line with previous data (Mazzi et al., 2014), the present results show that both healthy participants and a patient with a complete lesion of the ipsilateral V1 experienced phosphenes induced by TMS of IPS and that IPS-phosphenes have a higher phosphene-threshold and were signalled faster than those elicited by occipital TMS in healthy participants (see also Fried et al., 2011).

The novelty of the present paper relies on the use of a EEG–TMS interactive co-registration approach (Miniussi and Thut, 2010), which gave us the opportunity to directly measure the spatio-temporal dynamics of the cortical reactivity and connectivity (Ilmoniemi et al., 1997; Miniussi and Thut, 2010) due to the presence or absence of a phosphene and to draw some conclusions regarding the role of the occipital and parietal cortices during the emergence of awareness. Given the poor spatial resolution of the EEG technique, especially when performed with a low number of electrodes, rendering source localization analysis (e.g. LORETA) unreliable, the investigation of the exact source of neural activity in specific brain regions (e.g. a gyrus, sulcus or subcortical structure) goes beyond the scope of the present work. Instead, we adopted a surface-source imaging approach (the SCD analysis), which provided us with an approximation of the local current density flowing perpendicularly to the scalp, which is a reliable analysis even with the present number of electrodes (Luck, 2014, p. 165). By focusing on the different time windows of activity correlating with the presence/absence of a phosphene, we could identify both early gatekeepers of the awareness of phosphenes and late consequences of the ignition induced by previous neural activity.

In healthy participants, the awareness of phosphenes induced by occipital TMS correlated with activity in centro-temporal sites at an early phase (~70 ms after TMS) and in occipital sites at a late phase (starting at ~320 ms after TMS). Conversely, the awareness of phosphenes induced by parietal TMS correlated with activity in centro-parietal sites at an early phase (~70 ms after TMS) and in occipital sites at a late phase (starting at ~280 ms after TMS). Moreover, in the second experiment, IPS-TMS of the lesioned hemisphere in a patient with a complete lesion to the ipsilateral V1, showed that the awareness of phosphenes correlated with activity in parietal sites at an early phase (~80 ms after TMS), spreading to centro-parietal sites at ~165/170 ms after TMS. The activity induced by TMS does not extend to any later phase indicating that the late phase of activity in occipital sites found with healthy participants is not necessary for the awareness of the parietal phosphenes to emerge. Therefore, these data show different topographic maps and different time-courses of the phosphene present/absent effect depending on the cortical area being stimulated.

The different spatio-temporal dynamics of phosphene perception for the two stimulated sites in healthy participants and the differences found between healthy participants and the hemianopic patient for parietal phosphenes give us the opportunity to advance possible answers to the questions stated in the introduction about the “where” and “when/how” of the emergence of perceptual awareness.

Previous data (Mazzi et al., 2014) and the present paper tested the hypothesis that neural activity along the dorsal stream could have access to perceptual awareness. Here, we found that direct stimulation of IPS induces the perception of phosphenes and that this effect has its onset in the parietal cortex at ~70–80 ms after TMS. This result could point to a role of the parietal cortex as a local early gatekeeper of awareness. It could, however, be surmised that parietal phosphenes are generated in other visual areas (such as V1 or areas along the ventral stream) strongly connected to IPS. For this hypothesis to be tenable, one should find early neural activity correlating with the awareness of IPS-phosphenes in occipital or temporal areas. With respect to the involvement of V1, we found that neural activity correlating with phosphene perception can be detected in the occipital cortex of healthy participants only at a late time window after TMS of IPS. Moreover, in line with a previous report (Mazzi et al., 2014), we showed that a patient with a complete lesion to the ipsilateral V1 can still perceive phosphenes induced by IPS-TMS and that no activity at occipital sites (ipsi- and contra-lateral to TMS) can be detected at any time windows of the entire epoch, thus ruling out any contributions of feedback to V1 as a necessary mechanism for awareness to emerge (Zeki and ffytche, 1998; ffytche and Zeki, 2011). Similarly, with respect to the involvement of other areas along the ventral stream, we found no early activity in temporal sites after IPS-TMS neither in healthy participants (for whom SCD topographic maps are compatible with parietal generators) or the hemianopic patient (where no activity was found at temporal sites along the entire epoch), thus ruling out the possibility that the ventral stream, via its strong connections with the dorsal stream, could have contributed to the emergence of awareness of the IPS-phosphenes. Taken together, the results obtained with TEPs elicited by IPS-TMS show that IPS is an early and independent generator of phosphenes.

The role of the occipital and ventral cortices need to be further discussed in relation to the generation of phosphenes induced by TMS of V1/V2. In the present paper we found that occipital phosphenes correlated in healthy participants with an early onset of the phosphene present/absent effect in temporal sites. In line with a previous report (Taylor et al., 2010), occipital sites' activity

correlating with phosphene perception can only be found in a late time window. Unfortunately, the cited paper did not investigate the phosphene present/absent effect in sites other than the occipital cortex, thus rendering it difficult to determine a direct comparison between these and the previous results in terms of possible early activity in temporal sites correlating with occipital phosphenes. Interestingly, activity along the ventral stream has been found recently by combining TMS with fMRI (Halko et al., 2013). In this paper, phosphenes were induced by TMS of V1 and hemodynamic responses were measured. In line with the present results, the authors found neural activity correlating with the TMS pulse at both the lower left V1 (the targeted area) and lateral occipito-temporal cortex. Unfortunately, temporal resolution of fMRI is very poor, thus rendering it difficult to gather the temporal order of activations in occipital and temporal sites.

The kind of spatio-temporal dynamics found in the present paper could thus signal that temporal cortex is the early gatekeeper for the awareness of occipital phosphenes, in line with the notion that activity in higher-order visual areas along the ventral stream is best correlated with visual awareness than V1 per se (Rees et al., 2002). Activity in the occipital cortex would then represent a consequence of the feedback spreading from the temporal cortex, despite not having a causal role in the emergence of the phosphene perception. In line with the idea of a functional cross-talk between early and late visual cortices not having a causal role in awareness, are the results of parietal phosphenes in the hemianopic patient: no late (i.e. after 170 ms to TMS pulse) phosphene present/absent effect is found for any cortical sites. This evidence could be explained as the lack of activity in V1 due to the brain lesion i.e., neural activity in the parietal cortex, although causing feedback to V1 due to its anatomical connections, cannot receive the second sweep of activity from V1 and thus no late effects can be found in the parietal cortex.

Taken together, the results obtained with TMS of occipital and parietal sites, given the lack of early activity in occipital sites correlating with the awareness of phosphenes, can dismiss the causal role of V1 as an early gatekeeper of awareness. Indeed, early activity can be found only at parietal and temporal sites after TMS of parietal and occipital sites, respectively. However, a question still remains open as to the existence of a common site for the awareness of phosphenes, contributing to explain the summed activity found at temporal and parietal cortical sites. Given the lack of power of the present paper in being conclusive in finding the source of the EEG signal, only some speculations can be provided. Previous findings have shown that subcortical structures, such as the lateral geniculate nucleus (Kastner et al., 2006) can serve as an early gatekeeper in the control of visual attention and awareness. Future studies should therefore investigate the possibility that this might be the case not only for visual stimuli presented to the eyes but also for visual percepts generated by direct cortical stimulation by means of TMS. In the present paper, however, we have shown that the SCD topographical maps are different for the two stimulation sites, thus suggesting different subcortical generators (Vaughan, 1982; Michel et al., 2004) for occipital and parietal phosphenes. A finer arrangement of electrodes should be used in future studies to strengthen our conclusions that occipital and parietal phosphenes are generated by different areas. Alternatively, an optimal, although more technically demanding, candidate for this investigation and for overcoming the limitations of the present results and obtaining a finer localization of the brain sources of the awareness of occipital and parietal phosphenes, would be event-related optical imaging (Gratton and Fabiani, 2010; Wolf et al., 2008). Future studies could thus consider the possibility to study phosphene perception using this technique which, thanks to its high temporal (< 10 ms) and spatial (~ 1 cm^3) resolution, would add to the present results, providing a better understanding not only on the timing but also on the exact neural structures involved in the perception of occipital and parietal phosphenes.

The present data seem to be in favor of the proposal (Moutoussis and Zeki, 2002; Beauchamp et al., 2012) that local processes in high-order visual areas, specifically, the temporal and parietal cortex, could serve as early gatekeepers in which activity generates the emergence of conscious visual percepts (i.e. reflecting the correlates of "phenomenal awareness"; Block, 1996). In this respect, late activity found with healthy participants in the occipital or frontal cortex could be considered as the consequence of the "ignition" (Fisch et al., 2009) engendered in the temporal or parietal cortex (i.e. reflecting the correlates of "access awareness"; Block, 1996). However, in the literature there is a strong debate on the early vs. late correlates of visual awareness. Several event-related potential (ERP) studies have shown that the neural processes directly correlating with consciousness occur in the relatively early time window (e.g. Bachmann, 2009; Koivisto and Revonsuo, 2010; Railo et al., 2011) whereas other researchers have concluded that the neural processes directly correlating with visual awareness occur later (e.g. Dehaene and Changeux, 2011; Salti et al., 2012). The inconsistent results present in the literature render it unclear which time windows should be considered to correlate with the neural processing for generating consciousness (NCC), which correlate with the preceding processes (NCC-pr) and which with the consequences (NCC-co) of conscious perception (Aru et al., 2012). In this respect, it is important to note that the timing at which a neural process occurs cannot per se be informative but specific experimental manipulations need to be adopted (Aru et al., 2012). As an example in a recent paper (Pitts et al., 2014), the authors orthogonally manipulated visual awareness and task relevance and they found that late effects (i.e. the P300 component of ERPs) had to be considered as reflecting post-perceptual processes (NCC-co), not visual awareness per se (NCC). In the present paper, we had the opportunity to study a patient with a complete lesion to the ipsilateral V1 and we found that the neural processes correlating with the awareness of phosphenes remained confined in an early phase (with two peaks at $\sim$70–80 ms and $\sim$165–170 ms after TMS). Importantly, no effect was present in a late phase, despite the patient being aware of the presence/absence of the phosphenes. This piece of evidence, given the lack of late effects in patient SL but the presence of awareness, should imply that the late activity found with healthy participants in occipital and frontal areas as reflecting post-perceptual processes (NCC-co) and not visual awareness per se. Indeed, if the late activity is a proper neural correlate of awareness, no awareness should be possible without it. Conversely, it remains unclear whether the early activity found in both healthy participants and patient SL reflects only the NCC or a combination of NCC and NCC-pr. Unfortunately, the present experiment is neutral in this respect, both because no manipulation of the NCC-pr was adopted (Aru et al., 2012) and because a direct comparison between TEPs and ERPs latencies was not possible, given that in the former the entire subcortical pathway processing real stimuli was lacking, thus leaving us without any clues about the nature of neural processes preceding visual awareness when the percept was directly generated in the cortex. It would therefore be of great interest for future studies to adopt specific experimental manipulations to disentangle NCC-pr from NCC in phosphene perception.

An interesting aspect of the present results relates to the presence of visual qualia induced by TMS of the IPS that can be obtained also in the absence of a functioning V1. As discussed elsewhere (Mazzi et al., 2014; Silvanto, 2015), the presence of visual qualia in hemianopic patients has already been documented after TMS (Silvanto et al., 2007, 2008). Differently for previous reports, however, the present data and those obtained by Mazzi et al.

(2014) show that the stimulation of the sole damaged hemisphere in SL (and of patients SL and AG in Mazzi et al., 2014) was capable of inducing phosphenes in the blind field (see Silvanto, 2015 for a thoughtful discussion on the dissimilarities among the different studies). In a similar respect, a final point that deserves some consideration relates to the role of the primary visual cortex in vision. Indeed, a lesion to the primary visual cortex abolishes the ability to consciously perceive external visual stimuli. If, as the present data seem to suggest, V1 is not necessary for the perception of parietal phosphenes one should try to explain why V1 seems to be essential for normal vision. An interesting discussion can be found in a very recent review by Silvanto (2015, but see also Silvanto, 2008). In his view, of which we agree, one should consider the role played by a V1 lesion as affecting not only the functioning of V1 itself but that of all the visual areas in the hierarchy of visual processing. In this respect, a lesion to V1 would make the entire visual cortex, not just V1, still capable of low-level visual functions but not of maintaining perceptual awareness.

In conclusion, the present data show that temporal and parietal cortices, at least under the present circumstances, can serve as different local early gatekeepers of perceptual awareness in the human brain and that activity in the occipital cortex, although being relevant for perception in general (Koivisto et al., 2010; Silvanto, 2008, 2015), is not part of the neural bases of perceptual awareness (Crick and Koch, 1998).

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