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STUDYING HEMISPHERIC ASYMMETRIES IN VISUALLY RESPONSIVE AREAS: A TMS-EEG STUDY

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ABSTRACT

The last decades of neuroscientific research have seen a gradual flourishing of studies regarding the neural correlates of human consciousness, with evidence from perceptual studies and theoretical models progressively trying to elucidate the brain dynamics responsible for awareness to emerge. However, despite of the ever-increasing number of studies in the field, many aspects are still waiting for clarification.

One example of this, in the field of visual awareness, regards the possible hemispheric asymmetry in the neural mechanisms giving rise to visual experiences. In fact, it is known by now that areas located along both the classically defined ventral stream (associated with "vision for perception") and along the dorsal stream (the "vision for action" stream) can elicit visual percepts – in the form of phosphenes – when stimulated via transcranial magnetic stimulation. However, until now a direct comparison between the two hemispheres in the neural dynamics giving rise to these visual percepts has never been done.

With this work, therefore, we tried to shed light on possible differences between the two hemispheres in two cortical areas associated with either one of the two streams: we stimulated the early visual cortex (Experiment 1) and the posterior parietal cortex (Experiment 2) of both hemispheres to elicit phosphenes and compare the associated EEG activity. In both cases we found a clear hemispheric difference, with a left hemisphere showing an early local activation, followed by a more widespread ignition of neural activity; the right hemisphere, on the other side, displayed a later activation mainly localized over central electrodes. These results, consistent across the two experiments, point to the existence of distinct neural mechanisms in the two hemispheres for perceptual awareness.

The last part of this work is dedicated to better understand the functioning of transcranial magnetic stimulation, a stimulation technique commonly used in cognitive neuroscience. In spite of its widespread diffusion, the specific influence of some stimulation parameters is not completely understood. To shed some light on this aspect, we stimulated three premotor cortical targets in close proximity, each at three different coil orientation (0°, 45° and 90° respect to stimulated site). Our

aim was to disentangle the effect of coil orientation and slight coil transitions on the elicited TEP response. Our preliminary results seem to suggest that both factors have an influence, with orientation being the most influential factor: specifically, an orientation perpendicular to that of the stimulated gyrus seems to be able to elicit the strongest and most reliable response.

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Part A – Investigating hemispheric differences in the mechanisms of perceptual awareness

1. INTRODUCTION

1.1 The enigma of consciousness

Consciousness has always been a deep and challenging mystery for a wide array of disciplines. However, it's only in these last decades that neuroscience has started tackling this topic, trying to answer the "hard problem" about the roots of consciousness [1] with the formulation of scientific hypothesis and experimental designs able to address it. In particular, an increasing amount of effort has been dedicated to disentangling the neural correlates of consciousness (NCC), i.e. those brain structures and/or brain activity patterns that directly correlate with what is currently consciously experienced [2]. One of the main obstacles that has for long hindered research in the field is lack of a proper definition of consciousness; this has led to the birth of a number of different theories, each trying to focus on a specific aspect of conscious experience. Currently, such theories can roughly be divided into two main areas. Some of them are proposing explanatory mechanisms for differences in states of consciousness, identifying in a modification of the functioning state of the brain system the cause for changes in conscious states [3,4]. On the other side, another field is trying to elucidate the nature of the subjective content of experience (the so-called "qualia"), shedding light on what are the brain structures and activations actually responsible for what we are perceiving [5,6].

1.2 The two-streams hypothesis

In this latter field, vision has been the most extensively studied sensory modality. There is currently a wide range of theories trying to clarify the role that different visual areas play in visual awareness. The first macroscopic distinction refers to the difference between a dorsal and a ventral stream in the visual system [7,8]. Specifically, the dorsal stream, projecting from the occipital striate cortex to the posterior parietal areas, would be responsible for the sensorimotor transformations necessary to visually guide action and movements, while the ventral one, extending from the occipital striate cortex to the inferotemporal cortex, is the oneresponsible

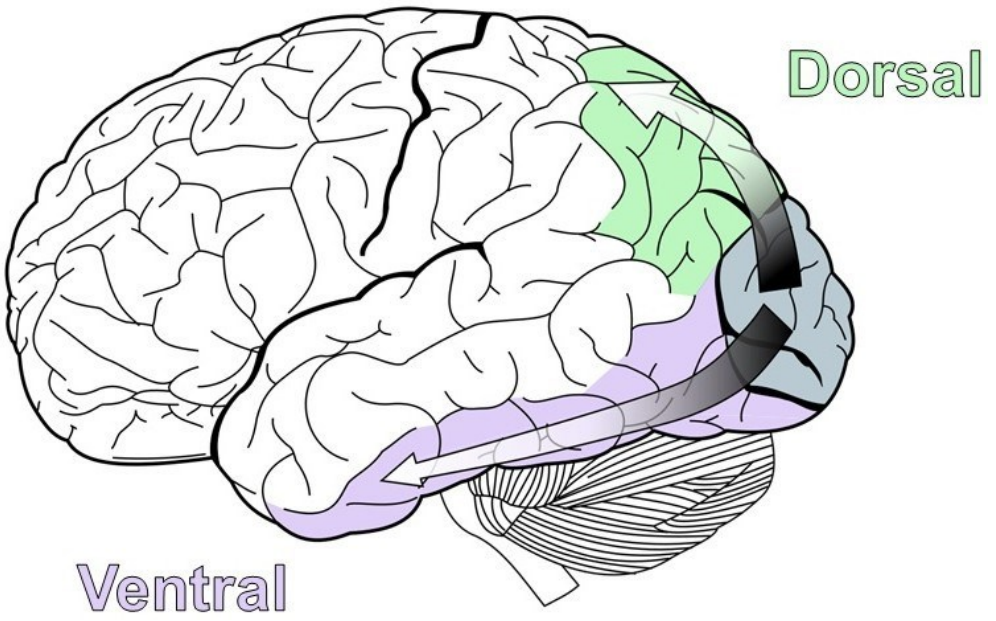

Figure 1.1 Visual representation of the two visual streams.

for perceptual identification and recognition of objects. For example, when we reach out to grasp an object, we usually have a complete visual experience of our hand and arm moving, of the shape and color of the target object, of the details of the environment in which it is placed, and so on. This visual awareness stems, following the model, from activity in the ventral stream. On the other side, this ventral processing has no causal role in real-time visual guiding of motor behavior: this is solely performed by dorsal stream processing, which is in charge of visually monitoring motor behavior. The same visual inputs that we experience thanks to the ventral stream are then used by the dorsal stream to provide guidance to our actions. One of the tenets of this theory, therefore, is that only the former stream is responsible for visual perception, while the dorsal one can operate in such a way that its neural output never reaches consciousness. For example, studies conducted on brain patients suffering from visual form agnosia – a rare condition that consists in the inability to distinguish different shapes or to report the orientation or size of presented items – have shown that these participants, while totally unable to report anything about the geometrical properties of a presented object, can nonetheless interact with it in a proficient and functioning way, thanks to a vast repertoire of preserved movements and gestures perfectly tailored to the very same geometrical properties they are incapable of distinguish [9–11]. Another fMRI study, conducted

on healthy participants, has shown that the presentation of masked stimuli (therefore unconscious) elicited a significant activation in the dorsal stream; most importantly, this activation was not significantly different from that recorded when the stimuli were consciously perceived. On the other side, large differences in ventral stream activity were found depending on the awareness or unawareness of the stimulus [12].

1.3 The role of feedback to V1 in visual perception

Other theories have targeted in an even more specific and deep way the role of specific areas of the ventral stream with regards to visual awareness. One of the most influential ones, originally proposed by Lamme and colleagues [13–15], identified the primary visual cortex and specifically feedback projections to V1 as the key mechanisms for the emergence of visual perception. Specifically, the neural feedforward activity following the appearance of a stimulus could be compared, although infinitely more complex, to that of a reflex arc: the sensory input coming from the occipital visual cortex is processed throughout the brain, undergoing increasingly complex levels of analysis that end with the execution of a motor output as an appropriate response to the stimulus. However, no part of this feedforward processing *per se* is conscious. Once the independent extraction of features and objects in a feedforward fashion is over, visual – and not only visual – neurons start then to influence each other's activity in a complex interaction of horizontal and feedback connections. This kind of activity, deeply modulated by the context and affected by the ongoing activity in connected neurons, is considered fundamental for the emergence of visual perception. A wealth of results, obtained in different experimental settings, seems to point to the fact that this recurrent activity is relevantly linked to conscious experience. For example, it seems to be abolished in anesthesia, while feedforward activity is not [16,17]. Moreover, manipulating visual consciousness is shown to influence EEG activity around 200 ms – a latency range known as visual awareness negativity (VAN) – which is a time range classically associated with recurrent visual processing [18]. These and other results seem to go in the direction of a role of recurrent visual processing in early visual areas, especially V1 [15].

1.4 Disentangling the true neural correlates of consciousness

Further experiments and studies, however, have shown that, while it is possible that activity in V1 may actually play a role in visual perception, it may not be the actual correlate of conscious visual experience [19]. When we talk about brain activity associated with consciousness, it is important to keep in mind that it can have one of three different functions [20]. First of all, brain activity could constitute a neural prerequisite for consciousness. This means that a specific brain process is necessary for a certain conscious experience to arise: if the process had not happened, we would not have had the corresponding conscious experience. However, such activity is not the neural instantiation of the conscious experience *per se*: it just constitutes a necessary condition for this latter to happen.

The second part that neural events can have with regards to perceptual awareness is that of neural consequences of conscious experience; that is, these activations are a result of having a conscious experience. Some of them may be potentially problematic to disentangle from the activity directly causing consciousness: in fact, there is the case that any conscious experience may always trigger some content-invariant neural consequences, irrelevant to the actual experience but nonetheless always following it. Even if this were the case, though, these latter neural events would not constitute the direct neural substrate of consciousness.

Finally, the final option is that the considered brain process is the actual neural substrate of the conscious experience. This has a twofold meaning: not only this neural activity is directly responsible for a specific percept, but it is also causally sufficient to elicit that percept. In other words, this neural substrate "is" the conscious experience.

1.5 V1: Actual correlate or just a prerequisite?

It has been known for a long time that any damage to V1 determines a region of blindness in the corresponding part of the visual field [21]. However, little is known about the specific role that V1 plays in visual awareness: its activity is just a necessary prerequisite for consciousness to emerge, or it constitute the actual substrate of conscious experiences?

Indeed, the existence of a phenomenon such as "blindsight" – i.e. the ability of some patients suffering from lesions on V1 to detect, localize and discriminate stimuli presented in their blind field, despite of the lack of visual awareness for that portion of the visual field – seems to point to the fact that V1 is the direct brain correlate of visual awareness [22]. However, numerous reports across the years have revealed the existence of patients with V1 lesions that are not completely unaware of events happening in their blind fields. While still lacking any form of phenomenal content, this degraded form of awareness has been described as a feeling of something "happening" in the visual blind field, and called blindsight Type 2 – opposing to the previous kind of blindsight without any form of awareness whatsoever, called Type 1 [23].

Moreover, other V1 patients have reported the presence of visual qualia in their blind fields [24]. In fact, there are reports of visual qualia in the blind field of V1 patients coming from at least three different sources [25]. First, patients experiencing the so-called Riddoch syndrome are known to experience visual motion qualia in the absence of any other visual percept [26]; a second proof of visual percepts in the absence of V1 comes from the phenomenon of hemianopic completion, in which a visual stimulus presented to V1 patients across the vertical meridian (so that it is in both the sighted and the blind hemifield) is entirely perceived by some of them, despite of the partial overlap with their blind region [27]; finally, a visual stimulus presented across the sighted and blind field [28] or even just in the blind field [29,30] can induce an after image in the blind field.

While it is quite established that V1 activity is tightly linked with visual perception and, if not entirely, at least partly necessary for proper visual percepts to arise, many doubts still remain around the matter if V1 activity is sufficient or just necessary for visual awareness to emerge [19]. In other terms, research should try to elucidate if V1 is directly or rather indirectly, through the regulation of activity in higher visual areas, contributing to awareness.

1.6 Shedding light over the dorsal stream

In recent years, accumulating evidence has started to put in discussion the two-stream theory as well, particularly the strong separation between a conscious ventral

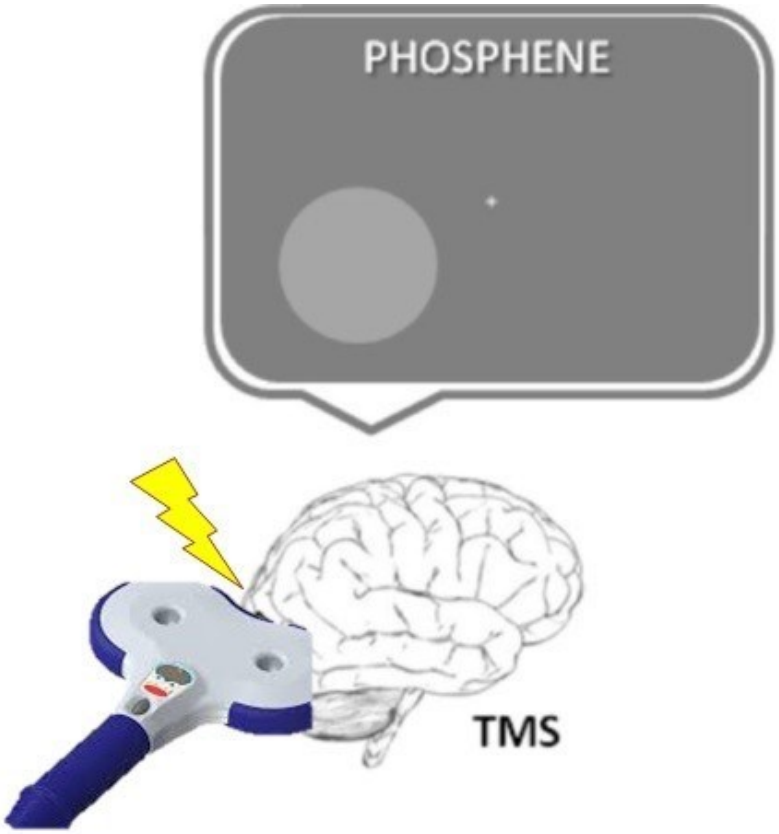

Figure 1.2 TMS applied over visually responsive areas (e.g., primary visual cortex) can elicit phosphenes.

and an unconscious dorsal stream that the model theorizes. In fact, it is well known that transcranial magnetic stimulation (TMS) applied over ventral occipital areas can induce the so-called phosphenes, i.e. visual sensations in absence of any light source determining them [31]. However, it is more recent the discovery that also dorsal parietal areas, classically thought to be unable to elicit conscious visual perceptions, can give rise to phosphenes as well when stimulated with TMS [32,33]. These results started to undermine the classical, rigid two-stream model, since they showed that even brain areas within the dorsal stream and classically associated with unconscious visuomotor processing and behavior guidance are capable of generating visual percepts.

Furthermore, studies combining TMS with EEG have started to investigate the neural correlates of these visual percepts. The results have shown that, despite sharing some phenomenological characteristics [32], the specific features of phosphenes and the neural activity associated with them are strongly dependent on the stimulated site: occipital phosphenes have been characterized as more stable

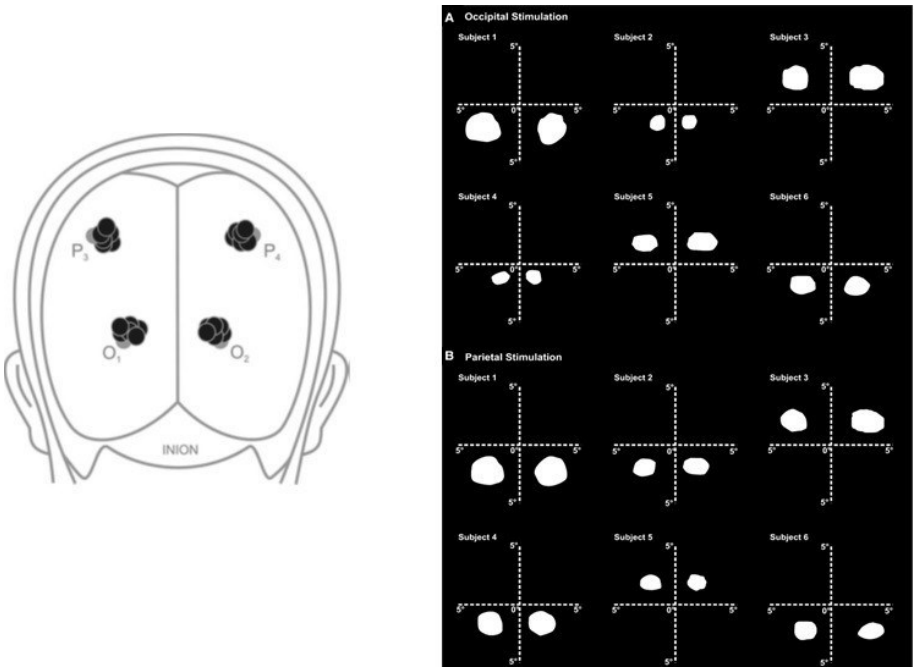

Figure 1.3 Stimulating IPS with TMS can elicit phosphenes in the participants, despite being an area located in the dorsal stream (adapted from [33]).

and well defined [34], and their perception has been shown to correlate with differential cortical activity in temporal areas [35]; on the other side, parietal phosphenes have been described as less vivid and bright [34], and their perception correlated with differential activity in the stimulated parietal location [35]. These phenomenological dissimilarities have also been confirmed in other experiments, in which TMS-induced phosphenes were used to reduce the visibility of presented stimuli. Occipital phosphenes, in fact, were able to reduce stimuli visibility at specific latencies, while parietal phosphenes were unable to do so [36]. Another aspect in which occipital and parietal phosphenes have shown differences is the respective interhemispheric transfer (IT) time [33]: using a Poffenberger paradigm, the authors compared the reaction times for a phosphene onset under the uncrossed or crossed hand-hemifield conditions. Results revealed a longer IT time for V1 phosphenes than for parietal ones, a difference due to the slower and sparser callosal connections subserving V1 compared to IPS. Differences have been reported also with regards to the oscillatory frequencies underlying the perception of phosphenes [37]. Occipital phosphenes were predicted by prestimulus power and phase in the alpha band, in accordance with previous literature [38,39]. However, the perception of parietal phosphenes was found to better correlate with beta band power (but not phase) [37]. Moreover, results from hemianopic patients have demonstrated that

parietal phosphenes perception is possible even in the absence of an intact ipsilateral V1 [34,35].

All of this evidence, taken together, goes in the direction of the existence of two different phosphene generators for occipital and parietal areas, undermining the tenets of the two-stream hypothesis: it is clear that parietal dorsal area can give rise to visual percepts as well, and such a clear-cut functional distinction between a perceptual, ventral stream and a visuomotor, dorsal stream is too simplistic.

1.7 The role of noninvasive brain stimulation techniques in studying visual consciousness

From the studies previously reported, it stems clearly that noninvasive brain stimulation techniques (NIBS), such as TMS, can potentially be powerful tools to disentangle the NCC prerequisites from actual substrates and consequences. In fact, NIBS allow to directly manipulate brain activity as an independent variable, and to evaluate the effects of this manipulation on conscious vision, allowing to draw causal conclusions on functional relevance [40]. For example, regarding visual consciousness, TMS has been applied over visual cortices, but also over parietal and frontal areas.

TMS could be applied on visual cortices to either elicit phosphenes (e.g. [41]) or to disrupt the processing of a visual stimulus (e.g. [42]). The first paradigm, in particular when TMS has been combined with EEG, has allowed to shed light on the phosphene-specific responses, while the second has contributed to inform models of visual awareness, thanks to the chronometric potential of TMS studies which permitted to identify early visual areas as either neural prerequisites or actual substrates of conscious vision.

The parietal cortex has been investigated as well with TMS with regard to visual consciousness. As reported above, studies have started to show that even parietal stimulation can give rise to phosphenes. Moreover, TMS has been consistently applied to mimic the effects of a “virtual” lesion, in order to mimic the effects on neglect syndrome or bilateral extinction [43]. Other studies have tried to clarify the functional role of parietal cortex on visual awareness, even though the distinction between attention and awareness has not always been clear (e.g. [44]).

Another TMS target in the study of visual awareness are frontal areas. In fact, these areas have been shown to be sometimes involved as NCCs, in particular in association with parietal areas [45]. Moreover, frontal areas seem to be involved in metacognitive processes associated to visual awareness, hosting therefore some perceptual neural consequences [46].

1.8 Aim of Experiment 1 and 2

In spite of a growing number of TMS-EEG studies being conducted in the field of visual awareness, many questions still remain open about the actual neural correlates of visual perception. One of them regards the possible asymmetricity of these mechanisms between the two hemispheres. In fact, despite both hemispheres being obviously able to give rise to visual percepts, many TMS studies involving phosphenes (e.g., [34,35]) have focused on the left hemisphere, on account of numerous reports showing that this hemisphere can evoke more reliable phosphenes than the right one [47,48].

However, a proper investigation on possible asymmetries in the generation of visual percepts may shed further light on the true neural correlates of visual experience, allowing to better identify the "where" and "when" the mechanisms of consciousness take place, and if they are unbalanced towards either one of the two hemispheres.

To do so, we performed two experiments aiming at comparing across hemispheres the evoked neural activity associated with phosphene perception. In the first one we stimulated left and right early visual areas, while in the second one we compared activations following left and right posterior parietal stimulation. In both experiments, TMS was applied at phosphene threshold (PT) intensity while EEG activity was simultaneously being recorded; this allowed us to disentangle the activity associated with conscious processing from the one associated with unconscious processing, and to compare activations across the two stimulation sites to check for possible hemispheric differences.

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2. EXPERIMENT 1

2.1 Introduction

A series of studies has begun, in the last few decades, to investigate possible differences between the left and right hemispheres in visual processing. Given the low-level of the involved functions, a hemispheric equivalence has often been assumed; however, a wealth of results has started to point to the fact that the two hemispheres are in charge of processing different characteristics of visual stimuli [1–5]: while the left contributes to analyze fine-grained visual details about the stimulus, the right is specialized in providing coarse, structural information.

It was reported by Robertson and colleagues [2] that lesions to right temporo-parietal regions affected the processing of the global level of hierarchical stimuli (i.e., Navon letters), while a damaged left superior temporal gyrus impaired local components analysis. Differences were also reported in healthy participants in the processing of local and global aspects of stimuli: Fink and colleagues, in a series of fMRI studies [3,4], reported that the left inferior occipital cortex oversees local elements processing, while the right lingual gyrus is responsible for global-level analysis of Navon letters. A work of Lux and colleagues further confirms this asymmetry [5]: they report that, when a local stimulus is shown within the left hemifield, the left posterior occipital cortex is activated. On the contrary, when a global stimulus is displayed in the right visual hemifield, there is an increase in neural activity in the right posterior occipital cortex. This result shows that stimulus information can travel through the corpus callosum to reach the hemisphere specialized for either local or global processing.

These two different levels of visual processing have recently been associated with low and high spatial frequencies (LSF, HSF), which are now considered as the visual characteristics responsible respectively for global and local representation [6–8]. The existence of a hemispheric asymmetry recalling the one for global and local processing [9] has been shown by numerous papers [10–12]. In an fMRI study by Musel and colleagues [13], for example, during a categorization task a left temporal predominance was found for HSF stimuli, while a right occipito-temporal

predominance was detected for LSF image processing. Another fMRI experiment aiming at comparing in the two hemispheres the spatial frequency bands [7] has shown that within the early visual areas there is a left predominance for HSF processing, and a right hemispheric predominance for LSF processing.

The existence of hemispheric differences after the presentation of visual stimuli has been suggested by other works. For example, Chokron and colleagues [14] have described how the lesion side influences the exact nature of the visual deficit in hemianopic patients, with performance impaired in different tasks depending on the lesion being located in the right or left occipital cortex. In an EEG study, Sanchez-Lopez and colleagues [15] have reported that a stimulus shown after either a valid or invalid cue elicited a differential time-frequency activity that depended on the hemifield of presentation.

Taken together, these results point towards asymmetric hemispheric processing of visual information, with the left hemisphere handling finer details, and the right hemisphere more involved in coarse visual analysis. However, most of these studies are based on imaging techniques, like EEG and fMRI, which can only establish correlational relationships. Therefore, we tried to directly check for lateralized differences in the visual system by stimulating the early visual areas of the two hemispheres with single-pulse transcranial magnetic stimulation (TMS), while recording the elicited activity with EEG. TMS when targeted over early visual areas can elicit phosphenes (i.e. visual percepts in the absence of any external stimulus) [16]. Indeed, the combination of these two techniques consents to noninvasively stimulate specific cortical areas and register the following spatiotemporal activations [17]; the recorded activity is directly caused by the TMS pulse. Therefore, eliciting phosphenes from both hemispheres can help us to assess the potential differences between the two hemispheres in generating visual percepts, shedding light on possible hemispheric differentiations in perceptual spatiotemporal dynamics. Asymmetries in the electrophysiological activations linked to phosphenes would strongly suggest that the two hemispheres possess lateralized differences in their spatiotemporal mechanisms responsible for visual perception.

2.2 Materials and Methods

Participants

Twenty-two right-handed volunteers (13 females, mean age 24.09±4.05), with normal or corrected-to-normal vision, were recruited for the study and reimbursed for their participation. We excluded from the analysis data from four participants because of either technical issues or excessively long reaction times (RTs). We obtained from the participants written informed consent according to the 2013 Declaration of Helsinki. The local Ethics Committee approved the experimental protocol.

We screened participants with a safety questionnaire (adapted from [18]) about the risk factors associated with TMS, and none reported any contraindications.

MRI image acquisition

Each participant underwent MRI with a 1.5 Tesla Philips scanner. A whole-brain high-resolution 3D T1-weighted image with magnetization-prepared rapid acquisition gradient echo (MPRAGE) (TR 7.7 ms/TE 3.5) was acquired.

Transcranial magnetic stimulation protocol

Single-pulse TMS were delivered via a 70 mm figure-of-eight coil connected with a Magstim Rapid2 system (maximum output 3.5 T, Magstim Company Limited, Whitland, UK). The TMS coil was placed tangentially to the surface of the scalp, with the handle pointing upward to avoid unspecific neck muscles activations.

We functionally detected stimulation sites through supra-threshold phosphene induction around electrode positions O1 (left hemisphere) and O2 (right hemisphere) of the 10-20 EEG system [19]. We used Neuronavigation based on individual MRI images (SofTaxic, E.M.S., Bologna, Italy and Polaris Vicra, NDI,

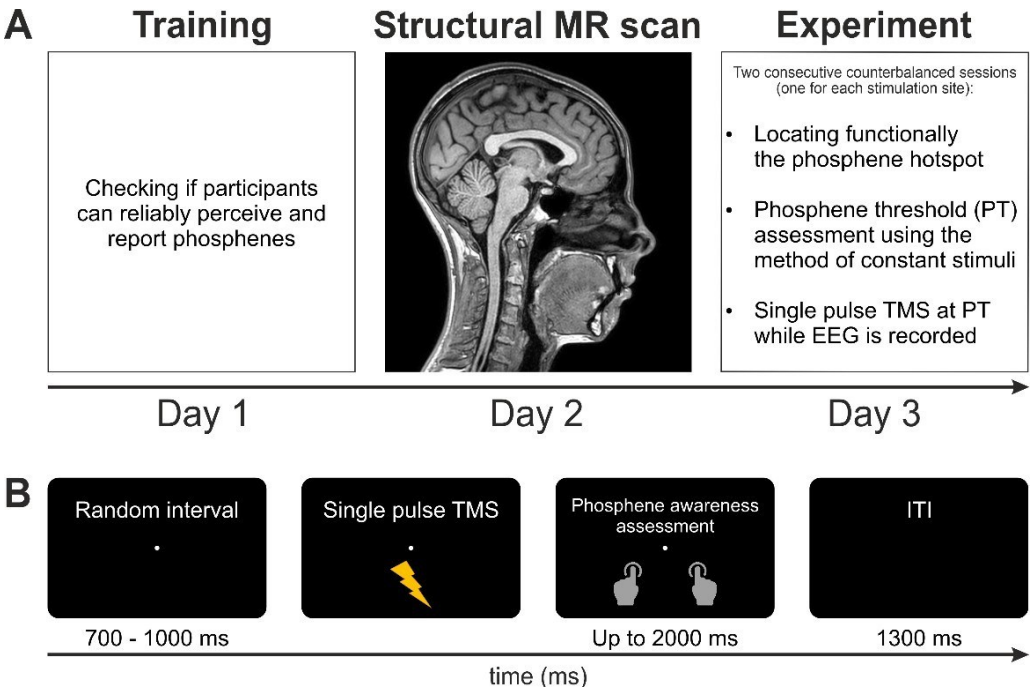

Figure 2.1 Experimental procedure and trial structure. **A**: Outline of the experimental procedure for each participant. **B**: Outline and timing of each stimulation trial.

Waterloo, Canada) to constantly control the focus of stimulation and possible coil displacements within a 2 mm accuracy threshold.

To determine the individual phosphene threshold (PT) for the two stimulation sites, the automatic procedure of the "Method of Constant Stimuli" (MOCS) [20] was employed. First, we functionally identified the hotspot for each of the two stimulation sites. The PT was then assessed by means of a computerized MOCS version: seven TMS intensities were randomly used (ranging from 60% to 78% of MSO, with increases in steps of 3%). We delivered seven pulses for each intensity, for a total of 49 pulses, and for each of them participants had to report the eventual phosphene presence. Data were fitted with a cumulative Logistic psychometric function via a maximum likelihood criterion using the Matlab Palamedes toolbox (http://www.palamedestoolbox.org). From the resulting function, we took the intensity at which participants perceived phosphenes in 50% of trials as the PT and it was used as stimulation intensity in the experimental phase.

Experimental procedure

We tested participants in a dark room. They sat in front of a monitor with their head secured in a chin rest to keep their eyes aligned with the central fixation point.

Participants were instructed to maintain their fixation on the fixation point for the whole experiment.

Before the experiment, each participant was tested during a training session for the perception of genuine phosphenes, and specific criteria had to be satisfied (Fig. 2.1A) [20]. After having been tested, participants underwent an MRI scan necessary for neuronavigated TMS.

During the experimental sessions, we administered single-pulse TMS at PT intensity to left or right occipital cortex while recording EEG. We counterbalanced the order of the two stimulation sites across participants. Each trial began with a random interval comprised between 700 and 1000 ms, followed by a TMS pulse. After each pulse, participants could report the presence or absence of a phosphene for the next 2000 ms by pressing respectively m (right hand) or z (left hand) keys on a keyboard, followed by 1300 ms of intertrial interval (Fig. 2.1B). Each participant underwent two consecutive sessions – one for each stimulation site– of 360 pulses each, divided into 6 blocks of 60 trials.

EEG recording and preprocessing

A TMS-compatible EEG equipment (BrainAmp, Brain Products GmbH, Munich, Germany) was used to register EEG activity (BrainVision Recorder), in combination with a Fast'n East cap with 59 TMS-compatible Ag/AgCl pellet pin electrodes (EasyCap GmbH, Herrsching, Germany) which was placed following the extended 10-20 International System. We used additional electrodes as online reference (RM), ground (AFz) and to monitor horizontal and vertical eye movements. We kept electrode impedance below 5 KΩ.

We positioned a custom-made polystyrene C-shaped annulus over the target electrode to reduce TMS-related artifacts and enable EEG recording from the electrodes underneath the TMS coil [16].

The EEG signal was processed off-line using Matlab 2021b (Mathworks, USA) with the EEGLAB toolbox (version 2021.0, [21]) and the TMS-EEG signal analyzer (TESA) extension [22].

First, we segmented 1000 ms before and after the TMS pulse the continuous raw signal digitized at 5000 Hz. We demeaned Epoched data using the whole epoch and

we removed the TMS pulse artifact from -2 to 10 ms. It was then replaced with cubic interpolation to avoid ringing artifacts. We then downsampled the data at 500 Hz. We performed a first round of independent component analysis (ICA) [23] for each participant to remove the TMS artifact. We then bandpass filtered (0.1-100 Hz, zero-phase, fourth-order Butterworth band-pass) and band-stop filtered (49-51 Hz) data. With a second run of ICA we then screened for blinks, lateral eye movements, persistent muscle activity, and electrode noise. To improve component decomposition, we substituted interpolated data from -2 before to 10 ms after TMS pulse with constant amplitude values before each ICA and interpolated them again thereafter. We then re-referenced data to a point at infinity [24] through the REST toolbox [25], low-pass filtered at 40 Hz, and epoched from -100 to 500 ms. O2 datasets were then flipped, in order to overlap the stimulation sites in the two experimental sessions. We then appended datasets and downsampled at 250Hz; the TBT toolbox [26] automatically detected and rejected bad trials (extreme values thresholds: +/- 125 μV, improbability and kurtosis criteria for single channels: SD > 5, for global threshold: SD > 3, maximum slope allowed: 50 μV, and minimal R squared allowed: 0.3). Baseline correction was finally performed from -100 to 0 ms.

For each participant we computed the Local Mean Field Power (LMFP) [27] for the two hemispheres (ipsilateral vs. contralateral electrodes to the stimulation; midline electrodes were excluded from this analysis) in order to better characterize the TMS-evoked activity following the lateralized stimulation.

We also computed a modified version of the interhemispheric signal propagation (ISP) index, a measure previously used to evaluate interhemispheric cortico-cortical dynamics [28–31], for the two stimulation sites. Contrarily to previous applications, in which the ISP index was calculated only on the stimulated electrode or on a limited cluster of surrounding electrodes, here it was calculated for each pair of homologue electrodes, in order to obtain an overview of the interhemispheric dynamics of the whole scalp. For this reason, we will call this measure global ISP (gISP). We computed this index for each pair of homologue electrodes (excluding the vertical midline) by rectifying the averaged amplitude of TMS-evoked activity for each participant across five windows identified around the peaks of the LMFP:

12-24 ms, 24-48 ms, 48-92 ms, 92-124 ms, and 124-240 ms. For the electrodes contralateral to the stimulation site, we shifted the latency of the time windows by 4 ms, in order to account for interhemispheric transfer time [32]. Through computing the gISP on subsequent time windows, we could get an overview of interhemispheric dynamics at different points in time: in this way it was possible to monitor the relationship in homologue electrodes activity across time, and to see how it changed with the progressive departure from the TMS pulse. The gISP was then calculated according to the following formula:

gISP = TMS-evoked activity contralateral / TMS-evoked activity ipsilateral

26 gISP values were obtained, each corresponding to a pair of homologue electrodes, which were later averaged and compared across the two stimulation sites.

Statistics

We analyzed behavioral data with JASP [33]. First, we compared PT values of the two stimulation sites using a paired samples t-test. Then, we applied a cut-off procedure to exclude trials from the experimental sessions with RTs<150 ms or >3 SD. A 2x2 repeated-measures analysis of variance (ANOVA) with *stimulation site* (O1|O2) and *phosphene awareness* (present|absent) as within-subject factors was performed on the percentages of positive and negative answers in terms of phosphenes detection. Moreover, we performed a one-sample t-test to check if the percentages of detected phosphenes differed from 50%. We carried out another 2x2 ANOVA with the same factors as the previous one on RTs.

We analyzed TMS-EEG data with MATLAB custom scripts and LIMO EEG toolbox [34]. We compared LMFPs via a series of 2x2 ANOVAs with *stimulation site* (O1 | O2) and *hemisphere* (ipsilateral | contralateral to the stimulation) as within-subject factors for each time-point. We also performed a 2x5 ANOVA on gISP values, with *stimulation site* and *time window* as factors, with multiple paired t-tests used as post hoc analysis to check for possible effects on the interaction. To correct for sphericity, we used a Greenhouse–Geisser correction, and we employed False Discovery Rate (FDR) [35] for multiple comparisons when applicable. With regards to TMS-evoked potentials (TEPs), a 2x2 ANOVA with *stimulation site* and

phosphene awareness as within-subject factors was performed, followed by t-tests to disentangle the interaction between factors. We then temporally thresholded these results using temporal clustering so that only significant activations equal to or longer than 12 ms were considered.

2.3 Results

Behavioural results

Stimulation sites for each participant are shown in Figure 2.2A. The mean PT for the left stimulation site was obtained at 69% of MSO and at 68.8% of MSO for the right stimulation site (Fig. 2.2B). These two values were not significantly different [$t(17) = 0.450$; $p = 0.658$].

The mean percentage of phosphenes detected through the experiment were also tested for being significantly different from 50%: this is not the case for both stimulation sites [O1: $t(17) = -1.354$; $p = 0.194$; O2: $t(17) = -0.737$; $p = 0.471$], thus supporting our procedure for establishing PT and an equal distribution of trials between conditions. On average, a phosphene was reported in the 45.4% of trials when stimulation was over O1, and in the 47.8% of trials when stimulation was over O2. Performing a 2-way repeated measures ANOVA on percentages of phosphene awareness we found no significant main effects (*stimulation site*: [$F(1,17) = 0.000$; $p = 1$; $\eta_p^2 = 0.000$]; *phosphene awareness*: [$F(1,17) = 1.357$; $p = 0.260$; $\eta_p^2 = 0.074$]) nor interaction [$F(1,17) = 0.920$; $p = 0.351$; $\eta_p^2 = 0.051$](Fig. 2.2C).

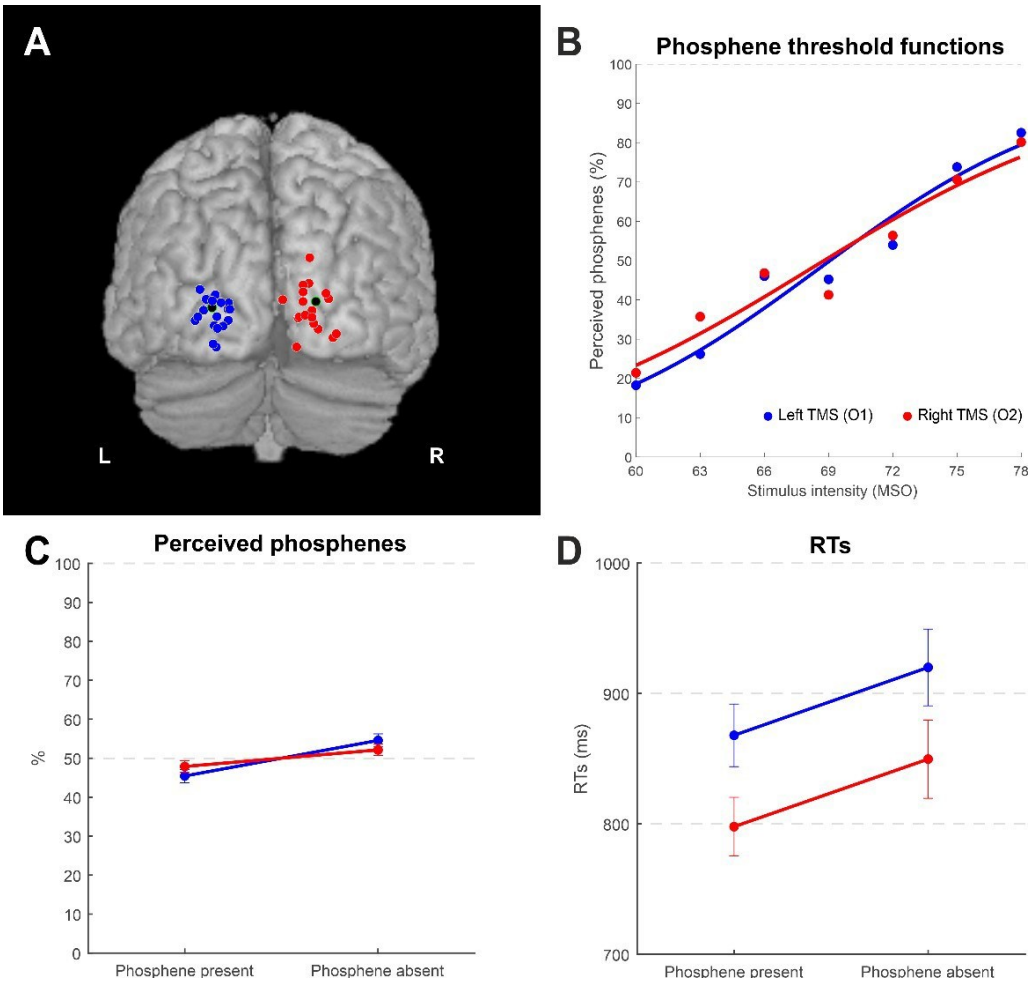

Figure 2.2 Stimulation sites, phosphene threshold functions and behavioral results. **A**: Stimulation sites for each participant in the two hemispheres corresponding to the individual hotspots eliciting phosphenes. Red dots represent left TMS, blue dots represent right TMS. Black dots respectively correspond to the O1 and O2 electrode positions. **B**: Average phosphene threshold functions for right and left occipital TMS. The intensity at which participants report a phosphene on 50% of pulses was selected as the stimulation intensity for the experimental sessions. Each dot represents the average number of phosphenes reported for that specific stimulation intensity; the fitted function illustrates the average phosphene threshold function across participants. **C**: Average percentage of reported phosphenes for the two stimulated sites. **D**: Average reaction times for positive and negative phosphene reports for the two stimulated sites. Error bars represent standard errors of the mean (SEM).

We detected through an ANOVA on RTs a significant main effect of stimulation site [$F(1,17) = 5.520$; $p < 0.05$; $\eta_p^2 = 0.245$] and phosphene awareness [$F(1,17) = 5.122$; $p < 0.05$; $\eta_p^2 = 0.232$] with RTs overall faster following phosphene perception and O2 stimulation, respectively. No interaction was found between the two factors [$F(1,17) = 1.985e\text{-}4$; $p = 0.989$; $\eta_p^2 = 1.167e\text{-}5$] (Fig. 2.2D).

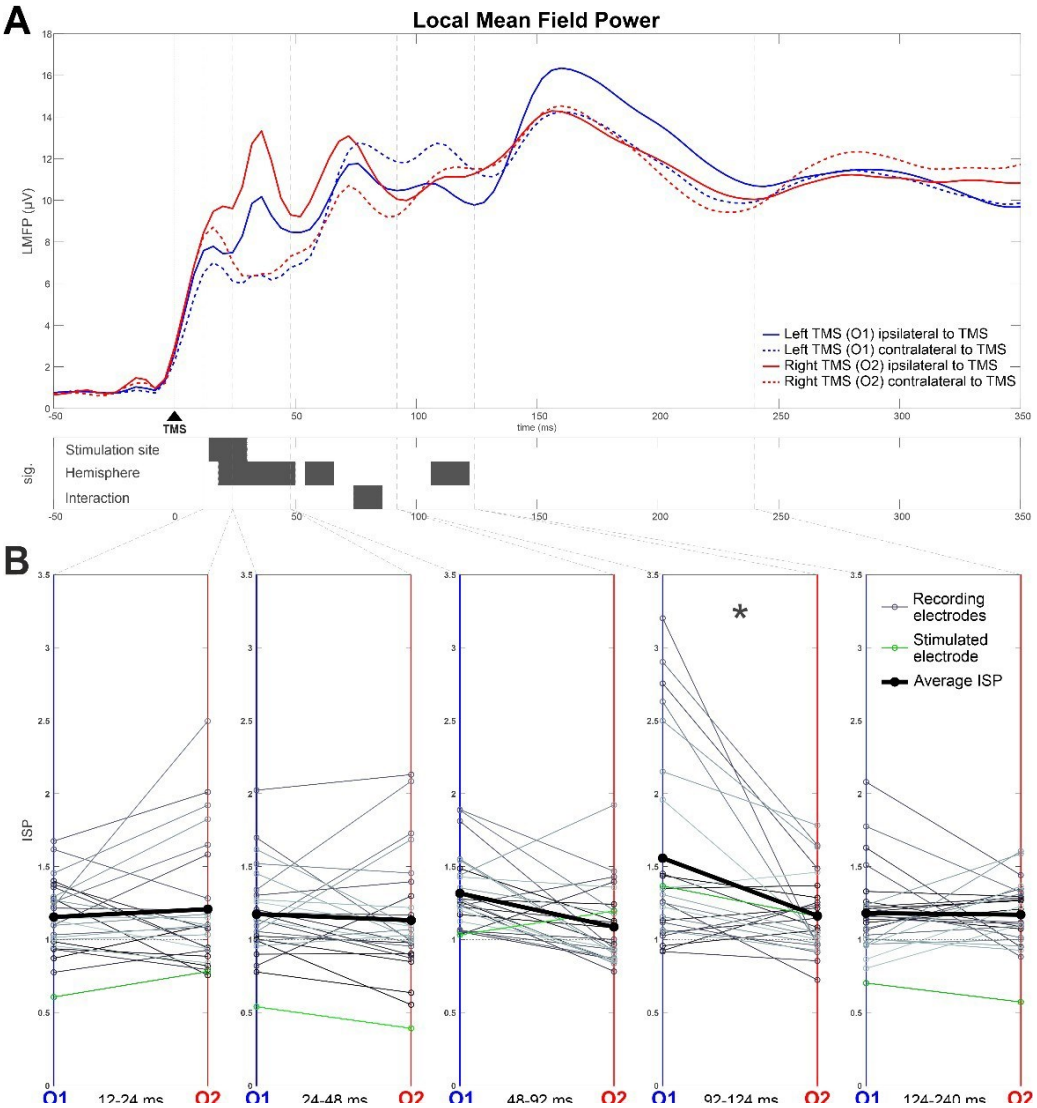

Figure 2.3 Local Mean Field Power and global Interhemispheric Signal Propagation Index (gISP). **A**: LMFP calculated separately for the electrodes ipsilateral and contralateral to the stimulation (vertical midline electrodes were not considered), for each stimulation site. Gray bars below the plot highlight significant results for each factor. **B**: gISP results for the five time windows selected around the peaks of LMFP. The two vertical axes of each plot represent, respectively, the gISP values corresponding to O1 (in blue) and O2 (in red) stimulation. The thick black line corresponds to the averaged gISP value across electrode pairs and participants. The green line represents the gISP value for the homologue pair of stimulated electrodes (i.e., O1 and O2). Each of the gray lines corresponds to the gISP value for a pair of homologue electrodes. Darker lines represent frontal electrodes, with the color becoming lighter and lighter formore posterior electrodes.

LMFP results

The analysis performed over LMFP data detected a significant effect of the stimulation site in the time range between 16 and 28 ms [all ps < 0.05]. Considering the hemisphere, significant differences were found between ipsi- and contralateral hemispheres to the stimulation site in the time range between 20 and 48 ms, 56 and 64 ms, and 108 and 120 ms [all ps < 0.05]. The interaction between the factors was found to be significant in the time range between 76 and 84 ms: post-hoc t-tests

detected a significant difference between the LMFP of the two hemispheres contralateral to the stimulation in the time range between 80 and 84 ms [all ps < .05] (Fig. 2.3A).

gISP results

With regards to gISP, the performed ANOVA have found a significant main effect of stimulation site [$F(1,17) = 4.768$; $p < .05$, $\eta_p^2 = 0.219$] and time window [$F(2.606,44.302) = 3.892$; $p < .05$, $\eta_p^2 = 0.186$]; also the interaction was significant [$F(2.816,47.876) = 3.889$; $p < .05$, $\eta_p^2 = 0.186$] (Fig. 2.3B).

We then performed a series of t-tests to disentangle the effects on interaction: they revealed that in the fourth time window (92-124 ms) left TMS elicited a higher gISP compared to right TMS [$t(17) = 2.9420$; $p = 0.0456$], while in the other time windows there was no differences between the two stimulation sites (W1 [$t(17) = -0.4783$; $p = 0.7851$], W2 [$t(17) = 0.7851$; $p = 0.5257$], W3 third [$t(17) = 2.3717$; $p = 0.0745$] and W5 [$t(17) = 0.2770$; $p = 0.7851$]) meaning that left TMS, compared to right TMS, elicited a higher amplitude in contralateral electrodes than in ipsilateral ones.

TEP results

To report significant TEP results, we identified temporal clusters around GMFP peaks. The two-way repeated measure ANOVA carried out on TEPs detected a significant effect [all ps<0.05] of *stimulation site.* We found three main clusters: the first in the 92-128 time window, comprising electrodes F4, F2, Fz, FC6, FC4, FC2, FCz, FC5, FC7, C6, C4, C2, Cz, C5, T7, CP4, CP2, CP1, developed mainly over right fronto-central electrodes; the second one in the 128-236 time window, comprising electrodes AF8, AF4, F8, F6, TP7, P5, P7, PO7, involved right frontal and left parietal electrodes; the last one in the 236-348 time window, comprising AF8, F8, T8, C6, TP8, P5, P7, was on fronto-parietal electrodes (Fig. 2.4A).

With regards to *phosphene awareness*, three clusters were highlighted: the first one in the 48-92 time window, comprising electrodes P3, P5, PO3, PO7, Oz, included left parieto-occipital electrodes; the second one in the 124-240 time window, comprising electrodes AF8, F8, F6, FT8, FC6, FC2, FCz, FC1, FC3, T8, C6, C2,

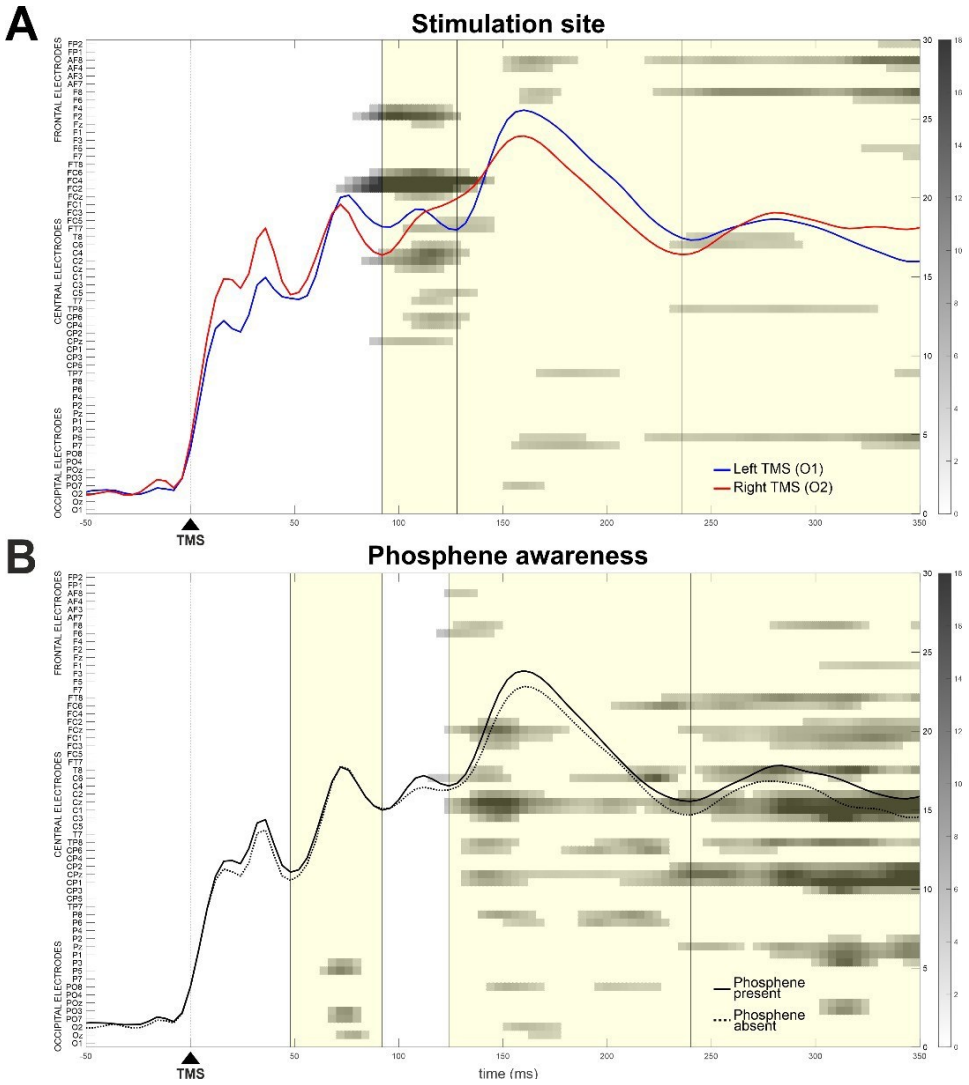

Figure 2.4 Results from the ANOVA conducted on TEPs: main effects of "Stimulation site" and "Phosphene awareness". **A**: Raster plot depicting for each time point and each electrode the significant differences (expressed in F-values) between "Left TMS" and "Right TMS". X and Y axis respectively represent time in milliseconds and electrodes (from posterior to anterior ones). The two lines superimposed represent the associated GMFPs (see right Y axis, expressed in μV), whose peaks were used to identify TEPs clusters. Right TMS data were flipped so that stimulation sites were overlapped in both experimental sessions. **B**: Raster plot depicting for each time point and each electrode the significant differences (expressed in F-values) between "Phosphene present" and "Phosphene absent" trials. Right TMS data were flipped so that stimulation sites were overlapped in both experimental sessions.

Cz, C1, C3, TP8, CP6, CPz, CP1, P8, P6, PO8, O2, Oz, involved mainly right fronto-central and parietal electrodes; the third one in the 240-348 time window comprising electrodes F8, F1, FT8, FC6, FC2, FCz, FC1, FC3, T8, C6, C2, Cz, C1, C3, TP8, CP6, CP2, CPz, CP1, CP3, P2, Pz, P1, P3, POz, PO3, developed mainly over right fronto-central and bilateral parietal electrodes (Fig. 2.4B).

Looking at the interaction, four different clusters were identified: the first one in the 12-24 time window, comprising electrodes AF4, F6, F4, F2, Fz, FC2, FT7, C5, T7, involving right frontal and left temporal electrodes; the second one in the

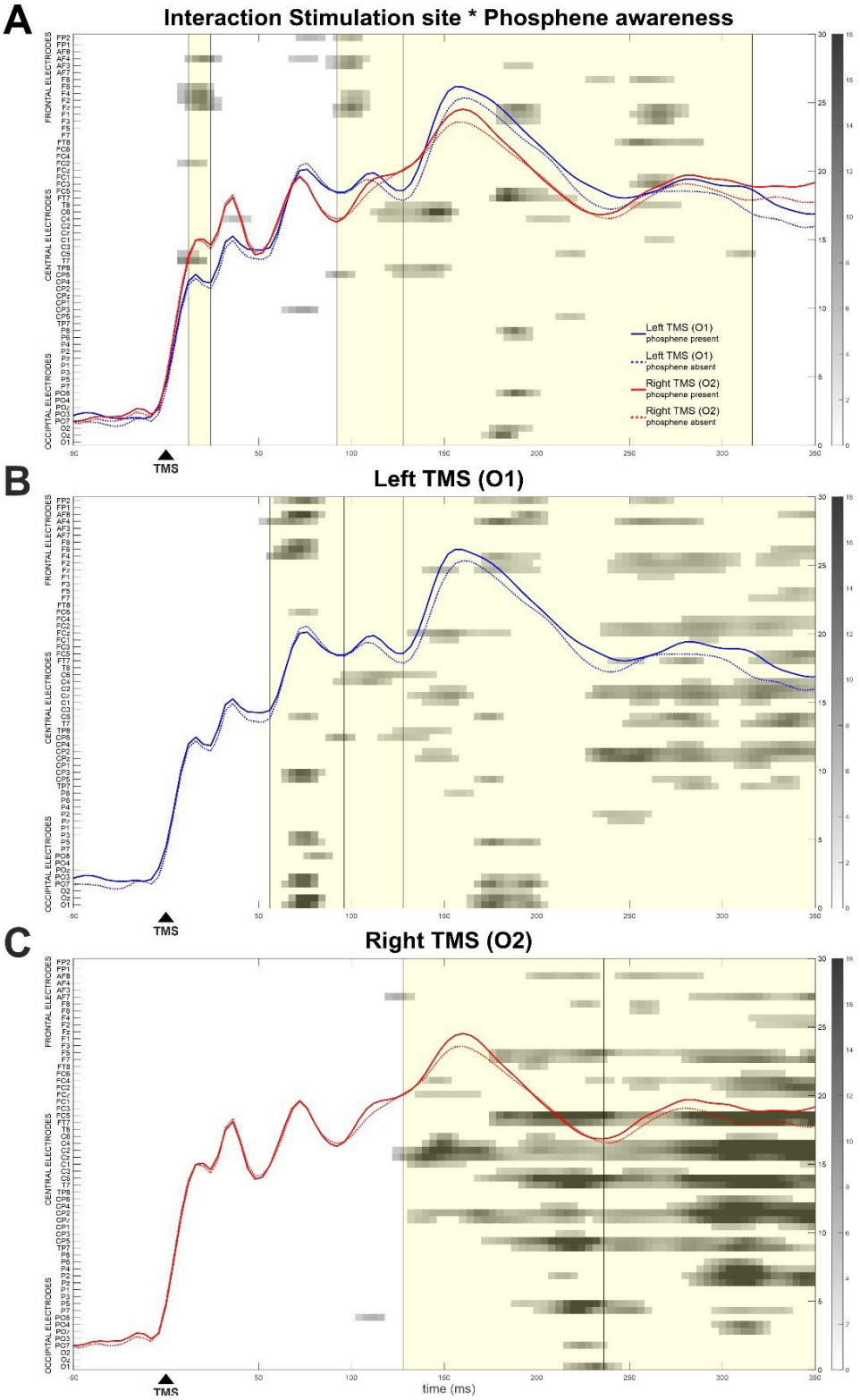

Figure 2.5 Results from the ANOVA conducted on TEPs: interaction and post-hoc pairwise comparisons. **A**: Raster plot depicting for each time point and each electrode the significant differences (expressed in F-values) between the four conditions. X and Y axis respectively represent time in milliseconds and electrodes (from posterior to anterior ones). The four lines superimposed represent the associated GMFPs (see right Y axis, expressed in μV), whose peaks were used to identify TEPs clusters. **B**: Raster plot depicting for each time point and each electrode the significant differences (expressed in t-values) between "Phosphene present" and "Phosphene absent" trials. **C**: Raster plot depicting for each time point and each electrode the significant differences between variables "Phosphene present" and "Phosphene absent" trials.

96-128 time window, comprising electrodes FP2, AF4, AF3, F4, F2, Fz, F1, T8, C6, C4, CP6, TP8, involving right frontal and central electrodes; the third one in the 128-240 time window included electrodes AF3, F8, Fz, F1, F3, FC5, FT7, C8, C6, C4, C5, TP8, CP6, CP5, P8, P6, PO8, O2, Oz, while the fourth cluster (240-316 time window) included F8, Fz, F1, F3, FT8, FC1, FC3, T8, C3, T7 (Fig. 2.5A). To disentangle the contribution of the different factors in the interaction, we looked at t-tests to check the different awareness-related activity in the two stimulation sites. When contrasting phosphene present vs. phosphene absent trials following O1 stimulation, four different clusters were identified: the first one in the 56-96 time window, comprising electrodes FP2, AF8, AF4, F8, F6, F4, FC6, C5, CP6, CP3, CP5, P3, P5, PO8, PO3, PO7, Oz, O1, involved left occipito-emporal and righ right frontal electrodes; the second one in the 96-128 time window, comprising electrodes C6 and C4, involved central electrodes; the third one in the 128-240 time window, comprising electrodes FP2, AF8, AF4, F4, F2, Fz, FCz, FC1, C6, C2, Cz, C1, C5, TP8, CP6, CP2, CPz, CP5, P8, P5, PO3, PO7, O2, Oz, O1, involved mainly right fronto-central and bilateral parieto-occipital electrodes, while the last cluster (240-348 time window), comprising electrodes FP2, AF8, AF4, F6, F4, F2, Fz, F1, F5, F7, FC4, FC2, FCz, FC1, FC5, FT7, C4, C2, Cz, C1, C5, T7, CP4, CP2, CPz, CP1, CP5, TP7, P2, Pz, involved bilateral fronto-central and centro-parietal electrodes (Fig. 2.5B).

Following O2 stimulation, two clusters emerged from the contrast of phosphene present vs. phosphene absent trials: the first one in the 128-236 time window, comprising electrodes AF8, F8, F5, F7, FT8, FC4, FCz, FC5, FT7, C6, C4, C2, Cz, C1, C3, C5, T7, CP4, CP2, CPz, CP3, CP5, TP7, P2, P5, P7, PO7, O1, involved bilateral fronto-central and parieto-occipital electrodes; the second one in the 236-348 time window comprising electrodes AF8, AF7, F8, F6, F5, F7, FT8, FC4, FC2, FCz, FC1, FC5, FT7, C6, C4, C2, Cz, C3, C5, T7, CP6, CP4, CP2, CPz, CP1, CP5, TP7, P6, P4, P2, Pz, P5, P7, PO4, POz, involved bilateral fronto-central and parietal electrodes (Fig. 2.5C).

2.4 Discussion

In this TMS-EEG study, the aim was to confront the spatio-temporal dynamics associated with visual perception between the two hemispheres. To do so, stimulation with TMS at threshold intensity was administered over the left and right early visual cortex to elicit phosphenes, i.e. conscious visual percepts known to be processed similarly to real external stimuli [19,36,37], while simultaneously recording EEG activity. Comparing LMFPs across the two stimulation sites showed that TMS elicits different patterns of electrophysiological activity as a function of the stimulation site and the hemisphere: while right occipital TMS caused early stronger activations, left occipital TMS determined late higher activity circumscribed to electrodes contralateral to the stimulation site.

The comparison of the gISP index determined by right and left TMS in five temporal windows revealed differences in how TMS-induced activity spreads across the electrodes during the time window ranging from 92 to 124 ms: after left TMS, activity tends to move contralaterally more than after right stimulation, pointing towards the existence of connectivity differences following right and left early visual areas stimulation.

With regards to phosphenes perception, TEPs analysis revealed the existence of hemispheric differences between the two early visual cortices. Phosphenes after left TMS are correlate with early occipito-parietal and frontal activity, while at later latencies central electrodes are progressively more involved; phosphenes after right TMS causes only late and central activations, with a marginal contribution of parietal and frontal areas.

Over the last years, only a handful of studies have focused on reporting hemispheric differences after administering TMS to early occipital areas. Among these, Garcia and colleagues [38] administered TMS over various homologue visual areas in both hemispheres, including the early visual cortex. Hemispheric asymmetries were reported in the magnitude and in the responses following lateralized V1 stimulation, especially at 40 ms after the stimulation. This resembles what we obtained with our LMFP results, where we report a difference from 16 to 28 ms between the two stimulation sites; even though it is slightly earlier than that reported by Garcia and colleagues, our result equally suggests the existence of early differences in the

electrophysiological response to TMS of early visual areas. This discrepancy between left and right V1/V2 emerges also in phosphene perception: phosphenes elicited by right and left TMS are associated with distinctive brain responses, suggesting that these differences might not be simply due to different connectivity, but actually play a role in visual perception.

Jarczok and colleagues, in another TMS-EEG study [39], concentrated on the interhemispheric differences in the brain response to lateralized TMS administration. In spite of not focusing, in their analysis, on the reported TEPs, it is nonetheless clear the presence of hemispheric differences in TEPs topographies evoked by either right or left stimulation. Differences are most evident between 80 and 100 ms over central and parieto-occipital electrodes, fading progressively as the epoch continues; our TEP results partially mirror these results, showing, in a similar time window, a difference over fronto-central electrodes as a function of the stimulation site. Comparisons between our results and those from Jarczok and colleagues, howeverm must be cautious despite the similarities: while our TMS targets were located around electrodes O1 and O2, they stimulated between electrodes P7/P8 and P11/12; this might account for the differences in TEPs topographies. Nevertheless, our results support the presence of differences in a time window around 100 ms after left and right TMS stimulation of early visual areas.

TMS-fMRI was also used to study hemispheric differences in connectivity. Ruff and colleagues [40] analyzed how occipital activity in the two hemispheres was affected by TMS over the right and left fronto-parietal cortex. Effects of frontal and parietal stimulation in the two hemispheres were markedly different. The activity in central visual field representations was decreased by both left and right frontal TMS, but only right frontal stimulation had an effect on the occipital lobe of both hemispheres, increasing activity in the peripheral field representations. With parietal stimulation these differences were even more evident: only right parietal TMS modulated activity in the visual cortex of both hemispheres. The predominance of the right hemisphere in visual processing might explain this hemispheric asymmetry [41], and thus explain the major capability in bilaterally influencing the occipital cortex. Our gISP results support this view: a higher contralateral activity after left TMS compared to right was found around 100 ms

after the stimulation, localized in particular over fronto-central electrodes. These imbalanced activations could be explained by feedforward connections between left occipital and right frontocentral areas, in charge of transferring visual stimuli to the competent hemisphere. In the comparison of these results, however, it is necessary to consider that they are obtained through different neuroimaging techniques, and that Ruff and colleagues have investigated top-down projections, our results supposedly go in the direction of a bottom-up connection, preventing us from drawing direct comparisons.

The electrophysiological correlates of phosphene perception are nother important point deserving consideration: to the best of our knowledge, this is the first study that systematically explored them across the two healthy hemispheres [42]. Bagattini and colleagues [16] recorded EEG activity correlating with phosphene perception after left occipital TMS stimulation. They showed the presence of bilateral differential activity over centro-temporal electrodes in a time window from 70 to 90 ms, followed by a late occipital activation starting around 320 ms until the end of the epoch. We found the earliest differential activity from 50 to 90 ms over frontal and parieto-occipital electrodes, followed by later more spread activity including frontal, central, and parieto-occipital electrodes. Bagattini and colleagues' results are in partial accordance with ours: both studies suggest the existence of an early time window, occurring between 50 and 100 ms, critical for phosphene perception after left occipital TMS. Differences in the EEG analysis pipeline, and the absence of individual MRI images in the study by Bagattini et al., which might have prevented in some participants the proper targeting of early visual areas, might explain differences in results. Taylor and colleagues reported TMS differential activity after right occipital TMS stimulation [43]. Two late activations spread over centro-parietal electrodes were reported: the first between 160 and 200 ms, and the second between 280 and 400 ms. Our results closely match those from Taylor and colleagues: we found our first cluster of differential activity between 130 and 240 ms, followed by a second one starting at 240 ms up until 320 ms; both are determined by a bilaterally widespread centro-parietal activation. Similarly to Taylor et al., phosphenes perception after right TMS in this study was related to late, central electrophysiological activity.

Taken together, our results point to the existence of different spatio-temporal dynamics resulting from the stimulation of the early visual cortices of the two hemispheres, with regards to phosphene perception. On the one side, right occipital stimulation bypasses any early posterior activation, activating a late bilateral central cluster which lasts basically unaltered for the duration of the entire epoch; on the other side, left occipital stimulation elicits earlier activity, initially limited to occipito-parietal and frontal electrodes, which gradually spreads towards fronto-central locations. This difference might be explained by the predominance of the right hemisphere compared to the left one in visual processing: while stimulation of the left hemisphere, eliciting weaker activity, allows to disentangle phosphenes conditions from an earlier time point, early activations in the right hemisphere are so strong that differences between the two phosphenes conditions (present/absent) cannot properly emerge (suggesting a "rooftop effect", supported also by our LMFP data showing higher early activity after O2 stimulation).

As pointed out above, different lateralized brain areas are considered responsible for processing different aspects of visual stimuli [44]; however, there is still debate about the electrophysiological mechanisms sustaining these different processes. The differences we detected after lateralized occipital stimulation, although not specifically addressed in this study, might represent activity patterns responsible for the functional differentiation of the two hemispheres.

In conclusion, in this study we showed that the brain response after early visual cortex stimulation differs between right and left hemispheres and that the activations associated with phosphene perception present a different pattern in the two hemispheres. Future studies should focus on investigating the possible presence of these asymmetric activations also in other phosphene sites [16,42,45].

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3. EXPERIMENT 2

3.1 Introduction

As already explained in the previous chapters, the study of visual awareness has given birth to a wealth of models trying to explain how a stimulus can give rise to a conscious percept. One of the most influential, originally proposed by Ungerleider and Mishkin [1] and successively updated by Goodale and Milner [2], is the two-stream hypothesis. In its formulation, this model suggested a modularity inside of the visual system, identifying two distinct streams of visual processing. While they both receive their input from the striate cortex, the pathways that follow are separate, with one ventral proceeding towards the inferotemporal cortex, and the other dorsal reaching the posterior parietal areas. The distinction is not only anatomical, but also functional: the ventral stream plays a significant role in the perceptual identification of objects, giving rise to the associated perceptual awareness; the dorsal stream, on the other side, seems to be prevalently linked with visuomotor guidance of actions and behavior, being responsible for the sensorimotor transformations necessary to proficiently interact with the environment, and whose processing is therefore constantly located below the consciousness threshold.

Recently, however, evidence has started accumulating that also areas located along the dorsal stream are potentially capable of eliciting visual percepts, undermining such a rigid division. Marzi and colleagues [3], in a TMS experiment, have been the first to find that stimulation of the intraparietal sulcus (IPS) can elicit phosphenes (i.e. visual percepts in the absence of any external visual stimuli), in a manner similar to what happens when stimulation is administered to early ventral visual areas. This finding, replicated in many other studies [4–8] has started to reveal that even the dorsal stream has the potential to produce conscious visual perceptions, and that a subdivision between a ventral stream in charge of visual awareness and a dorsal stream unconsciously managing visually guided behavior might be too straightforward.

Many of these studies, however, have focused on stimulating only one hemisphere, usually the left one. An aspect still lacking some clarification, therefore, is the existence of asymmetric differences between the two IPS with respect to the neural mechanisms associated with the generation of visual percepts. While the literature on hemispheric asymmetries in visual awareness is still quite scarce, the situation is different for visual attention. Attention is a research topic different from but strictly linked to that of visual awareness [9], whose neural substrates have been consistently placed in various areas of the parietal lobe, including IPS. Hemispheric asymmetries in the field of visual attention have been deeply investigated, to the point of becoming one of the core tenets of many attentional models.

For example, in Kinsbourne's opponent processors model – one of the most influential –, each hemisphere determines an attentional bias towards the contralateral visual hemifield, with the rightward bias determined by the left hemisphere being stronger than the leftward bias from the right hemisphere [10]; this potentially explains the more frequent appearance of phenomena like neglect and extinction after a rightward than a leftward parietal lesion.

This asymmetry, originally suggested by a wealth of neuropsychological evidence, has been later confirmed also by numerous TMS studies, showing how stimulating right IPS had a differential effect than stimulating the left one [11]. For example, Capotosto and colleagues [12] have interfered with repetitive TMS (rTMS) over both left and right IPS, to check for hemispheric differences in the allocation of spatial attention. Results revealed that rTMS over both hemispheres disrupted the lateralized alpha anticipatory modulation of the occipital visual cortex, but only when rTMS targeted right IPS a paradoxical synchronization of pretarget alpha rhythms and a subsequent bilateral deficit in target identification were detected. Moreover, Bien and colleagues [13] administered TMS over left and right IPS to mimic the neurophysiological syndrome of contralateral extinction, i.e. an impairment, usually following parietal lesions, in perceiving multiple stimuli of the same type presented simultaneously. They found that, while TMS on both sides resulted in contralateral extinction, only left hemifield extinction after right IPS stimulation is significantly worsened by a rival stimulus in the ipsilesional

hemifield. These results point to the existence of hemispheric asymmetries in the response of IPS to stimulation.

The aim of this work, therefore, is to determine if IPS asymmetries between the two hemispheres exemplified above for the attentional field can be detected also in the realm of visual awareness. To do so, we performed a TMS-EEG study stimulating left and right IPS at phosphene threshold (PT). The activity elicited from phosphene perception was then compared across the two stimulated sites, to detect possible hemispheric differences in the neural dynamics giving rise to visual awareness.

3.2 Materials and methods

Participants

Twenty-three right-handed volunteers (3 males, mean age 22.73±2.52), with normal or corrected-to-normal vision, participated in the study and were reimbursed for their participation. Written informed consent was obtained from participants according to the 2013 Declaration of Helsinki. The experimental protocol has been approved by the local Ethics Committee. Participants underwent a screening questionnaire (adapted from [14]) addressing risk factors associated with TMS; no participant reported any contraindications.

MRI image acquisition

We acquired MRI images from seven participants. They underwent MRI with a 1.5 Tesla Philips scanner at the Borgo Roma Hospital in Verona. We acquired a whole-brain high-resolution 3D T1-weighted image with magnetization-prepared rapid acquisition gradient echo (MPRAGE) (TR 7.7 ms/TE 3.5).

Transcranial magnetic stimulation protocol

Single-pulse magnetic stimulation via a 70 mm figure-of-eight coil connected with a Magstim Rapid2 system (maximum output 3.5 T, Magstim Company Limited, Whitland, UK). TMS coil was placed tangentially to the scalp surface, keeping the handle upward to avoid unwanted activations of neck and shoulder muscles.

We used a neuronavigation software (SofTaxic, E.M.S., Bologna, Italy) combined with a 3D optical digitizer (Polaris Vicra, NDI, Waterloo, Canada) to control for

possible coil displacements within a 2 mm accuracy threshold and to verify the stimulation target.

Location of stimulation sites was performed through supra-threshold phosphene induction within a circle of 2 cm in diameter centered on electrode P3 (left hemisphere) and P4 (right hemisphere) of the 10-20 EEG system [5]. Neuronavigation based on individual MRI images, when available, was used to constantly check that the focus of the stimulation was aimed at the IPS; when this was not the case, an MNI based template was employed in the neuronavigation software.

We used the "Method of Constant Stimuli" (MOCS) [15] to establish the individual phosphene threshold (PT) for the two stimulation sites. We took the intensity at which participants perceived phosphenes in 50% of trials as the PT and used it as stimulation intensity during the experiment.

Experimental procedure

Participants sat in a dark room in front of a 17in. LCD monitor at 57 cm. Their head were secured in a chin rest to keep their eyes aligned with the screen center, on which participants were instructed to maintain their fixation during the experiment. Before the experiment, we conducted a training session during which participants were tested for the perception of genuine phosphenes – identified by specific criteria (Fig. 3.1A) [15].

Participants had to report, for each TMS pulse, the presence or absence of a phosphene by pressing the keyboard keys *m* (right hand) or *z* (left hand), with the responses counterbalanced across participants. They underwent two consecutive sessions – one for each hemisphere, whose order of stimulation was counterbalanced – comprising six blocks of 60 stimulations each, for a total of 360 TMS pulses. They were stimulated at PT intensity while wearing earplugs reproducing white noise for auditory masking (Fig. 3.1B).

EEG recording and preprocessing

TMS-compatible EEG equipment (BrainAmp, Brain Products GmbH, Munich, Germany) was used to record EEG activity (BrainVision Recorder), in combination

with a Fast'n East cap with 59 TMS-compatible Ag/AgCl pellet pin electrodes (EasyCap GmbH, Herrsching, Germany) positioned according to the 10-20 International System. We employed additional electrodes as online reference (RM), ground (AFz) and to control horizontal and vertical eye movements. We kept electrodes impedance below 5 KΩ.

We positioned a custom-made polystyrene C-shaped annulus over the electrode targeted by TMS, to enable EEG recording from it and to reduce TMS-related artifacts [8].

The EEG signal was processed off-line with Matlab 2021b (Mathworks, USA), the EEGLAB toolbox (version 2021.0, [16]) and the TMS-EEG signal analyzer (TESA) extension [17].

The continuous raw signal, digitized at 5000 Hz, was cut 1000 ms before and after the TMS pulse. Demeaning was performed on epoched data using the whole epoch. The TMS pulse artifact was removed from -2 to 10 ms and replaced with cubic interpolation to avoid ringing artifacts. Data were then downsampled at 500 Hz. Independent component analysis (ICA) [18] was then used to remove the TMS artifact. Subsequently we applied on data a bandpass filter (0.1-100 Hz, zero-phase, fourth-order Butterworth band-pass) and band-stop filter (49-51 Hz). We performed a second run of ICA to check for blinks, lateral eye movements, persistent muscle activity, and electrode noise. Before each ICA round, interpolated data from -2 before to 10 ms were substituted with constant amplitude values and interpolated again thereafter to improve component decomposition. We then re-referenced data to a point at infinity [19] using the REST toolbox [20]. Finally, we low-pass filtered the data at 40 Hz and epoched them from -100 to 500 ms. O2 datasets were flipped in order to overlap the stimulation sites in the two experimental sessions. Datasets from both hemispheres were then attached and downsampled at 250 Hz. We used TBT toolbox [21] to perform detection and rejection of bad trials. Baseline correction was finally performed from -100 to 0 ms.

For each participant we then computed the Local Mean Field Power (LMFP) [22] for the two hemispheres, both considered as ipsilateral and contralateral to the stimulation; midline electrodes were not included in the calculation.

Statistics

JASP was used to analyze behavioral data [23]. Trials with RT<150 ms or >3 SD were considered outliers and excluded from further analysis. A 2x2 repeated-measures (ANOVA) with *site of stimulation* (O1 | O2) and *phosphene awareness* (present | absent) as within-subject factors was used to analyze RTs. Another ANOVA with the same factors was employed on the percentages of positive and negative responses; we then performed a one-sample t-test to check if these percentages differed from the 50% threshold.

MATLAB custom scripts and LIMO EEG toolbox were used for TMS-EEG data analysis [24]. A 2x2 ANOVA with *site of stimulation* (O1 | O2) and *laterality of stimulation* (ipsilateral | contralateral) as within-subject factors was carried out on LMFPs. We performed a 2x2 ANOVA with *stimulation site* and *phosphene awareness* as within-subject factors on TMS-evoked potentials (TEPs), followed by t-tests to study factors' interaction. We then thresholded results by applying a temporal filter that revealed only significant activations whose duration was at least 12 ms. The correspondence between significant TEP and GMFP activity was used to define clusters of electrodes.

3.3 Results

Behavioural results

The mean PT for the two stimulation sites was obtained at 74.5% of maximum stimulator output for P3 stimulation site, and at 75% for P4 stimulation site. These two values were not statistically different [$t(22) = -0.510$; $p = 0.615$].

The mean percentages of detected phosphenes were 37.2% after P3 stimulation and 33.6% after P4 stimulation (Fig. 3.1C).

A 2-way repeated measure ANOVA on the percentages of detected phosphenes found a significant main effect of *phosphene awareness* [$F(1,22) = 43.110$; $p < .001$; $\eta_p^2 = 0.662$], revealing that the percentage of detected phosphenes is significantly lower than the percentage of negative reports. Both *stimulation site* [$F(1,22) = 0.193$; $p = 0.665$; $\eta_p^2 = 0.009$] and interaction [$F(1,22) = 4.028$; $p = 0.057$; $\eta_p^2 = 0.155$] were not significant.

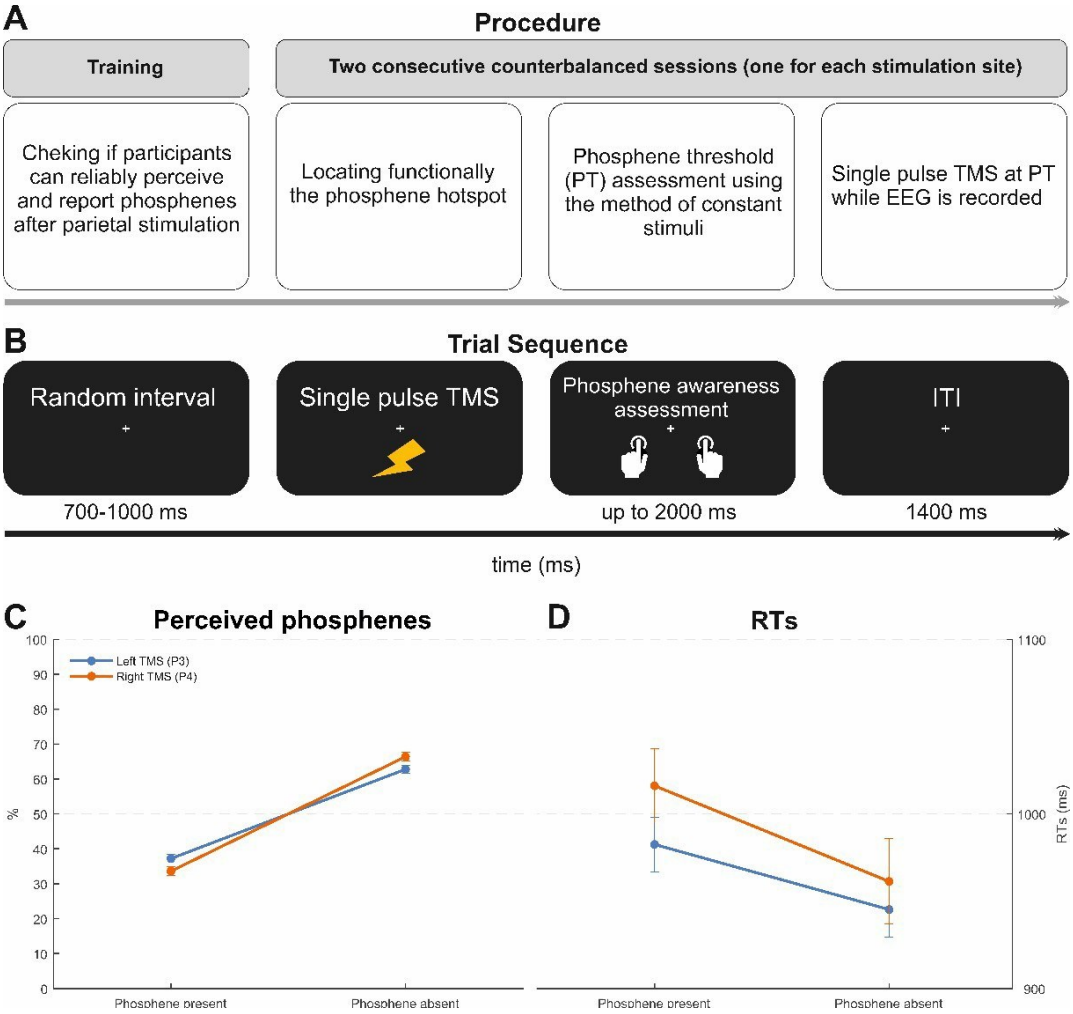

Figure 3.1 Experimental procedure, trial structure and behavioral results. **A**: Outline of the experimental procedure for each participant. **B**: Outline and timing of each stimulation trial. **C**: Average percentage of reported phosphenes for the two stimulated sites. **D**: Average reaction times for positive and negative phosphene reports for the two stimulated sites. Error bars represent standard errors of the mean (SEM).

A 2-way repeated measure ANOVA conducted on RTs showed a significant effect of *phosphene awareness* [$F(1,22) = 5.754$; $p < .05$; $\eta_p^2 = 0.207$], with positive phosphene reports (982 ms for P3 and 1016 ms for P4) being significantly slower than negative reports (945 ms for P3 and 961 ms for P4) (Fig. 3.1D). This might be explained by the nature of parietal phosphenes, which are often described as weaker and fainter than occipital ones [5]: participants might have needed therefore more time to realize that they had perceived something, increasing the amount of time needed to confirm a positive report. Neither the *stimulation site* [$F(1,22) = .394$; $p = 0.536$; $\eta_p^2 = 0.018$] nor the interaction [$F(1,17) = 0.641$; $p = .432$; $\eta_p^2 = 0.028$] were significant.

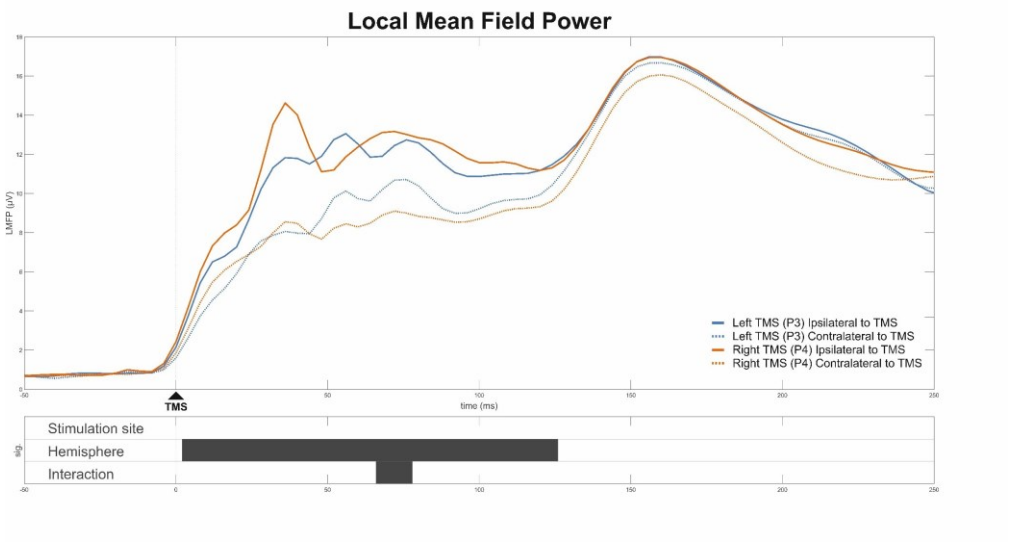

Figure 3.2 Local Mean Field Power (LMFP) calculated separately for the electrodes ipsilateral and contralateral to the stimulation (vertical midline electrodes were not considered), for each stimulation site. Gray bars below the plot highlight significant results for each factor.

LMFP results

The analysis performed over LMFP data detected a significant effect of the *stimulated hemisphere* (ipsilateral | contralateral) in the time range between 12 to 124 ms [all ps < .05]. No significant differences were found for the *stimulated site*. The interaction between factors was found to be significant in the 68 to 76 ms: post-hoc t-tests in that time interval revealed a significant difference in the ipsilateral and contralateral LMFPs for each stimulation site, with ipsilateral LMFP possessing a higher amplitude than contralateral LMFP for both P3 and P4 stimulation sites (Fig. 3.2).

TEP results

To report significant TEP activations, we identified temporal clusters based on GMFP peaks. A two-way repeated measures ANOVA on TEPs revealed a significant effect of *stimulation site* [all ps < .05]. We found three main clusters: the first one in the 46 – 64 ms time window, comprising electrodes F4, F2, Fz, F5, F7, FC6, FC4, FC5, FT7, C4, C5, T7, CP5, TP7, P7, involved mainly bilateral fronto-central and left temporo-parietal electrodes; the second, in the 64 – 96 ms time window, comprising electrodes AF7, Fz, F5, F7, FC6, FC4, FC5, FT7, C4, C5, T7, CP5, TP7, P7, POz, O2, involved again mainly bilateral fronto-central and left

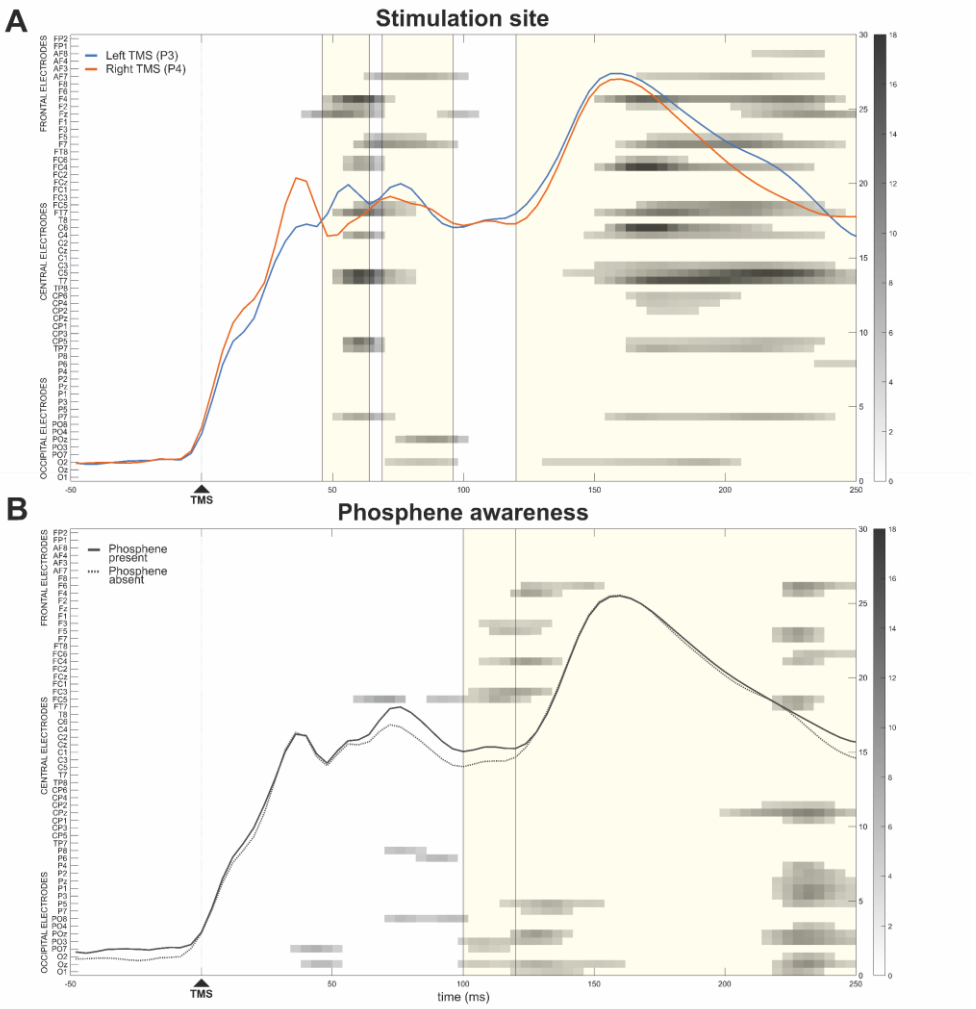

Figure 3.3 Results from the ANOVA conducted on TEPs: main effects of "Stimulation site" and "Phosphene awareness". **A**: Raster plot depicting for each time point and each electrode the significant differences (expressed in F-values) between "Left TMS" and "Right TMS". X and Y axis respectively represent time in milliseconds and electrodes (from posterior to anterior ones). The two lines superimposed represent the associated GMFPs (see right Y axis, expressed in μV), whose peaks were used to identify TEPs clusters. Right TMS data were flipped so that stimulation sites were overlapped in both experimental sessions. **B**: Raster plot depicting for each time point and each electrode the significant differences (expressed in F-values) between "Phosphene present" and "Phosphene absent" trials. Right TMS data were flipped so that stimulation sites were overlapped in both experimental sessions.

temporo-parietal electrodes; the third one in the 120 – 250 ms time window, comprising electrodes AF8, AF7, F4, F2, Fz, F5, F7, FC6, FC4, FC5, FT7, C6, C4, C3, C5, T7, CP6, CP4, CP2, CP5, TP7, P6, P7, O2, involved mainly bilateral frontal, central and parietal electrodes (Fig. 3.3A).

With regards to *phosphene awareness*, two different clusters were identified: the first one, in the 100 – 120 ms time window, comprising electrodes F3, F5, FC4, FC3, FC5, P5, PO3, PO7, Oz, involved mainly left frontal and parieto-occipital electrodes, the second, in the 120 – 250 ms time window, comprising electrodes F6,

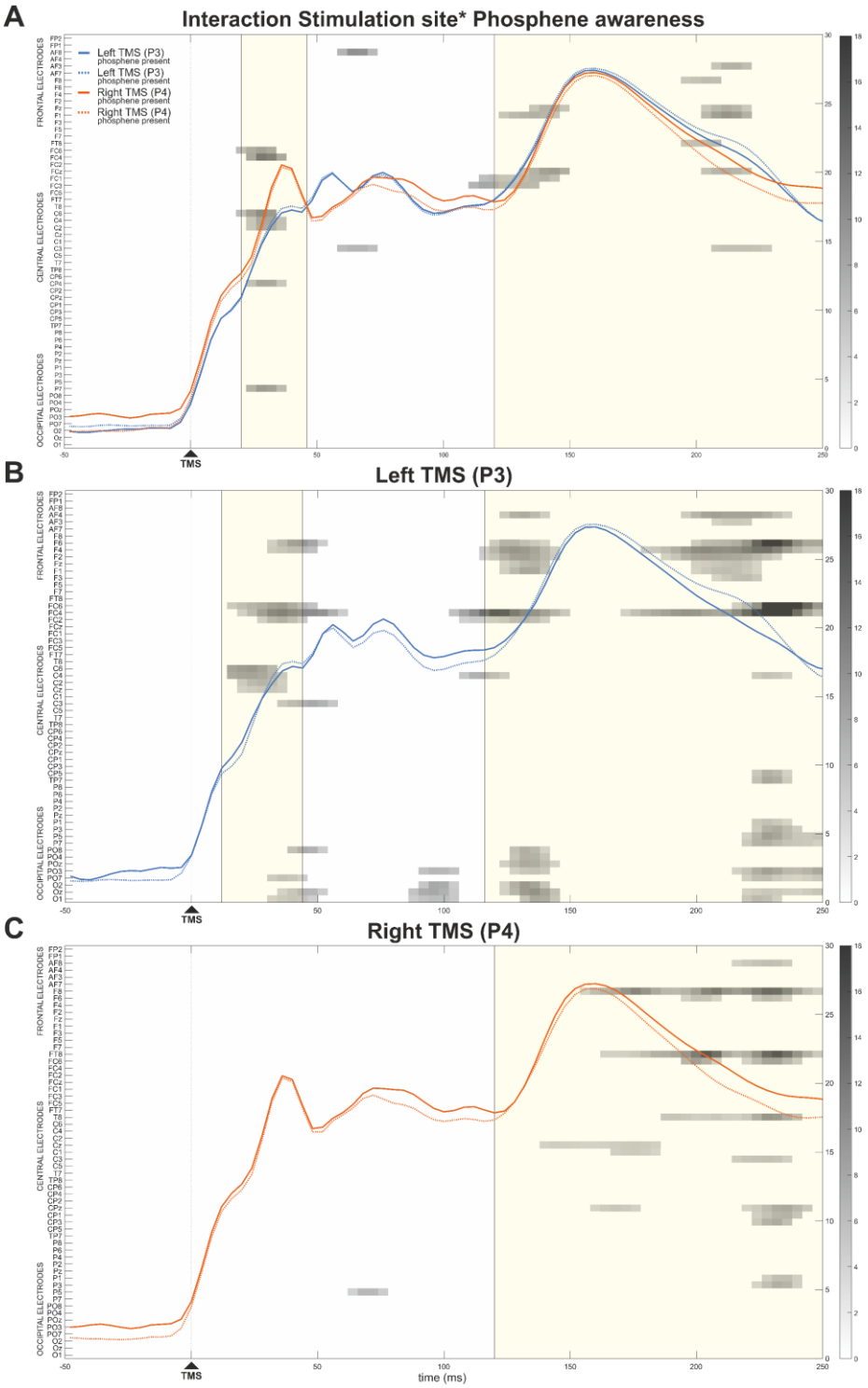

Figure 3.4 Results from the ANOVA conducted on TEPs: interaction and post-hoc pairwise comparisons. **A**: Raster plot depicting for each time point and each electrode the significant differences (expressed in F-values) between the four conditions. X and Y axis respectively represent time in milliseconds and electrodes (from posterior to anterior ones). The four lines superimposed represent the associated GMFPs (see right Y axis, expressed in μV), whose peaks were used to identify TEPs clusters. **B**: Raster plot depicting for each time point and each electrode the significant differences (expressed in t-values) between "Phosphene present" and "Phosphene absent" trials. **C**: Raster plot depicting for each time point and each electrode the significant differences between variables "Phosphene present" and "Phosphene absent"trials.

F4, F3, F5, FT8, FC6, FC3, FC5, CP2, CPz, CP1, P4, P2, Pz, P1, P3, P5, PO4, POz,

PO3, O2, Oz, O1, involved bilateral frontal, central, parietal and occipital electrodes (Fig. 3.3B).

Considering the interaction between the two factors, we detected two clusters: the first in the 20 – 46 ms time window, comprising electrodes FC6, FC4, C6, C4, C2, CP4, P7, involved right frontocentral electrodes; the second in the 120 – 250 ms time window, comprising electrodes AF3, F8, F1, F3, FT8, FCz, FC1, FC3, C3, involved mainly left frontocentral electrodes.

In order to disentangle the contribution that the different factors gave in the interaction, we looked at the t-tests to check for the awareness-related activations in the two stimulation sites. When contrasting phosphene-present vs. phosphene absent condition after P3 stimulation, we found two cluster: the first in the 12 – 44 ms time window, comprising electrodes AF7, F6, F4, F7, FC6, FC4, FC2, FT7, C6, C4, C2, Cz, C3, CP4, CP2, PO8, PO7, Oz, O1, involved mainly right fronto-central and parietal electrodes; the second, in the 116 – 250 ms time window, comprising electrodes AF4, AF3, F6, F4, F2, Fz, F1, F3, FC6, FC4, C4, CP5, TP7, P8, P3, P5, P7, PO8, PO4, POz, PO3, PO7, O2, Oz, O1, involved bilateral frontal, central, parietal and occipital electrodes.

With regards to P4 stimulation, one cluster emerged from contrasting phosphene present vs. absent conditions: it was located in the 120 – 250 ms time window, comprising AF8, F8, F6, FT8, FC6, T8, Cz, C1, C3, CPz, CP1, CP3, P3, P5, involved mainly right fronto-temporal and left centro-parietal electrodes.

3.4 Discussion

The aim of the present TMS-EEG study was to shed more light on the spatiotemporal dynamics of the dorsal stream associated with visual awareness, and to detect possible hemispheric asymmetries. To do so, we administered TMS at PT intensity over right and left IPS, an area of the dorsal stream which is known for its ability to give rise to visual percepts in the form of phosphenes [3,5], while concurrently recording EEG. This allowed us to compare brain activity associated with phosphene perception with that resulting from a stimulation not eliciting any percept, and to see if these activations present any hemispheric asymmetry.

The comparison of the LMFP across the two stimulation sites revealed an effect of the laterality of the stimulation: electrodes ipsilateral to the stimulation presented a higher degree of variability compared to the ones on the contralateral side, which was a predictable result. This was further confirmed by looking at the disentangled effect of stimulation laterality for the two stimulation sites: for both left and right sites, in fact, ipsilateral electrodes showed a higher degree of variability compared to the contralateral ones.

With regards to TEPs, we found differential activations for the two stimulated sites, hinting at the existence of connectivity differences between left and right IPS. Unsurprisingly, differences in elicited activity were also found for phosphenes perception. In particular, when the effect of awareness was disentangled for the two stimulation sites, we could find hemispheric asymmetries in the activity elicited by phosphenes perception: in fact, left IPS phosphenes correlated with early fronto-central and parietal activity, followed at a later latency by a more widespread activation comprising frontal, central, parietal and occipital electrodes; on the other side, right IPS phosphenes were associated with a late activation, diffused over right fronto-temporal and left centro-parietal electrodes.

In the last years, a series of TMS studies, reporting the appearance of parietal phosphenes after IPS stimulation, have started to undermine the two-streams model for visual perception, which postulated a clear-cut separation between a dorsal, unconscious, stream, and a ventral, conscious, one [25]. In fact, many of these results have shown how parietal and occipital phosphenes are clearly differentiated in terms of brain sources [5] and neural spatiotemporal dynamics [7,8], pointing therefore towards the existence of distinct generators for these two perceptual phenomena.

Mazzi and colleagues [5] have shown that with TMS stimulation on IPS it is possible to elicit phosphenes in the blind field of hemianopic patients suffering from a complete destruction of primary visual cortex: the reported phosphenes were described and scored in a way similar to that of healthy control participants, and a psychophysical function created to describe the relationship between stimulation intensity and phosphene perception was similar to that obtained for healthy participants. These results prove that parietal phosphenes are a distinct percept from

occipital ones: they possess a different cortical generator, independent from feedback to primary visual cortex, and can be therefore studied in their own electrophysiological characteristics. This result confirms that the phosphene participants reported in our study were actually due to IPS stimulation, with no involvement of early visual cortex.

Up to this date, two other studies have tried to investigate the electrophysiological correlates of parietal phosphene perception: Bagattini and colleagues stimulated left IPS [8], while Samaha and colleagues probed right IPS[7]. More specifically, Bagattini and colleagues reported that phosphene perception after left IPS stimulation was associated with differential activity in the stimulated parietal area. In particular, they found two phases of activity correlating with visual perception: the first early one started at around 60 ms after the TMS pulse, while the second one, much later, started at around 210 ms. On the other hand, Samaha and colleagues report the presence of the first differential activity around 200 ms. Our results are at least in partial agreement with these studies. In fact, for left IPS phosphenes we found early differential activity, starting as soon as ~15 ms after the TMS pulse, followed by another burst of activations between 100 and 150 ms; on the other side, right IPS phosphenes correlated predominantly with activity arising no earlier than 150 ms, thus displaying a later onset for awareness-related activations. Even though the specific temporal intervals between our results and others' might not perfectly overlap, the idea of a left hemisphere in which perceptual differences start to emerge early and, on the contrary, a right hemisphere displaying awareness-related activity only later in time is consistent in these three studies, allowing us to pinpoint these hemispheric asymmetries.

Interestingly, this pattern seems to resemble the one we obtained in the previous occipital experiment, in which we found differences as well between the two hemispheres in the form of an earlier left hemisphere and a later right hemisphere. This is further confirmed by both Samaha's and Bagattini's studies, in which both authors stimulated occipital sites in addition: these latter show similar temporal dynamics to the ones found after parietal stimulation, with early left activations and late right ones. It seems therefore that the most striking differences, at least in a temporal perspective, are not due to the specific stimulated area, but rather on the

stimulated hemisphere, since the temporal dynamics we found seem to hold true for different phosphene areas located in the same hemisphere.

To our knowledge, this is the first study in the field of visual awareness that stimulated both IPS. Similar studies with bilateral IPS stimulation, however, have been conducted in the field of visual attention. One of the first TMS studies reporting hemispheric differential effects in stimulating IPS is the one conducted by Dambeck and colleagues [26]. In this study they reported that, while TMS applied over both IPS was able to transiently impair the attentional functioning of the parietal cortex, much stronger effects were obtained when TMS administered over right IPS. Another study from Cazzoli and colleagues [27], employing theta burst stimulation, showed a similar pattern of results, with right IPS stimulation typically sorting stronger behavioral effects on attention, and a left one unable to exert any influence.

Another series of TMS studies has tried to clarify the relationship between IPS and early visual areas, and how stimulating the former can exert an effect on the latter's activity. Ruff and colleagues [28], in a TMS-fMRI study, have shown that right and left IPS have a different influence on early visual areas: only right parietal TMS, in fact, was able to elicit strong BOLD changes in early visual areas, while left parietal TMS had no effect on them. This result suggests a right-hemisphere predominance in influencing visual perceptual areas, with right IPS being able to directly modulate activity in early visual cortices (but see also [29]). A different study going in a similar direction is the one from Koivisto and colleagues [30]. Starting from an experiment equally focusing on the parietal influence on early visual cortex but targeting just one hemisphere [31], in this study they stimulated both IPS and found that only right IPS was able to elicit both behavioral and electrophysiological effects: right IPS TMS influenced the visibility of presented Gabor patches, while left one had no effect on the participants' ratings; Moreover, right IPS TMS reduced the amplitude of the posterior N1 component, typically associated with recurrent visual interactions in ventral visual areas, in the time window 180-220 ms, considered critical for visual conscious perception. This result highlight once more the existence of hemispheric asymmetries in IPS when it comes to visual awareness, with a right IPS having a dominant role in influencing both behavior and neural

activity. Another study, investigating the role of IPS in inducing perceptual fading [32], found again a hemispheric difference, with right IPS TMS able to influence the hit rate of both visual hemifields, and left IPS TMS influencing only the contralateral visual field; again, this supports a right hemisphere predominance in visual attention and perception.

This wealth of results, investigating different neural aspects of cognition and perception, show nonetheless the existence of hemispheric asymmetries, both in connectivity and in function, between the two IPS; this asymmetry goes in the direction of a right, dominant IPS, whose stimulation is able to influence both behavior and remote visual areas, and a left IPS, more restrained and local in its influence. Our results, showing the existence of hemispheric asymmetries in the neural activity associated with parietal visual percepts, are in accordance with the framework of a dominant right IPS: as shown above, left IPS phosphenes elicit a differential activity from an early time point, while for right IPS phosphenes these differentiations emerge only at a later time: this might be due to a sort of "rooftop" effect, due to which processing of right IPS phosphenes determines a higher level of activation right from the start, with more time – and processing – needed for differential activity between conditions to emerge; on the other side, the differential activations associated with left IPS phosphenes can emerge earlier in time, due to the processing being performed by the "weaker" hemisphere.

Our study has further investigated the presence of hemispheric asymmetries in IPS, switching from the realm of visual attention to the one of perception. It also contributes to better define the neural correlates of parietal phosphenes, contributing to establishing the reliability of this phenomenon and its usefulness to study visual awareness. Further studies should focus on better defining, at a spatial level, the dynamics characterizing the appearance of these percepts, and possible hemispheric differences also in this regard.

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Part B – Studying the influence of stimulation parameters in TMS-EEG

4. INTRODUCTION

4.1 TMS-EEG: Principles and functioning

As shown in the previous chapters, TMS-EEG can be a very powerful tool for the field of cognitive neuroscience, particularly in the study of visual awareness. In spite of its widespread use, however, we are still quite far away from a complete and deep understanding of the effect that TMS stimulation can have on local and remote neural network dynamics, with the risk of being unable to distinguish between the actual brain response and confounding effects of the stimulation [1]. It is therefore necessary to improve our grasp of how TMS can shape our neural activity, so that researchers could profit the most out of this brain stimulation technique.

TMS-EEG is born from the combination of transcranial magnetic stimulation, a non-invasive brain stimulation technique, with electroencephalography [2].

TMS is based on the physical principle of electromagnetic induction, discovered by Faraday in 1831, which demonstrated that electric currents and voltages were induced only by a changing magnetic field, and not by a static one. This principle is exploited in TMS: when a pulse is given, in fact, a current flow with high voltage (1-2 T) and extremely short duration (1 ms) passes through the coil; when placed over the participant's head, this generates rapidly changing magnetic pulses that penetrate the scalp and skull, reaching the brain with negligible tissutal attenuation. These magnetic pulses induce eddy currents (i.e. secondary ionic currents) in the brain, that are able to penetrate neuronal membranes, resulting in an action potential or in an excitatory/inhibitory postsynaptic potential [3]. One of the main characteristics of TMS is that the magnetic field falls off rapidly with the increase of distance from the coil [4], so it is usually safe to assume that, unless the stimulation intensity is particularly high, the magnetic pulse activates neural elements at the surface of the brain, like in the cortex or in subcortical white matter. This strongly limits direct stimulation to the outer parts of the cerebral cortex located under the skull.

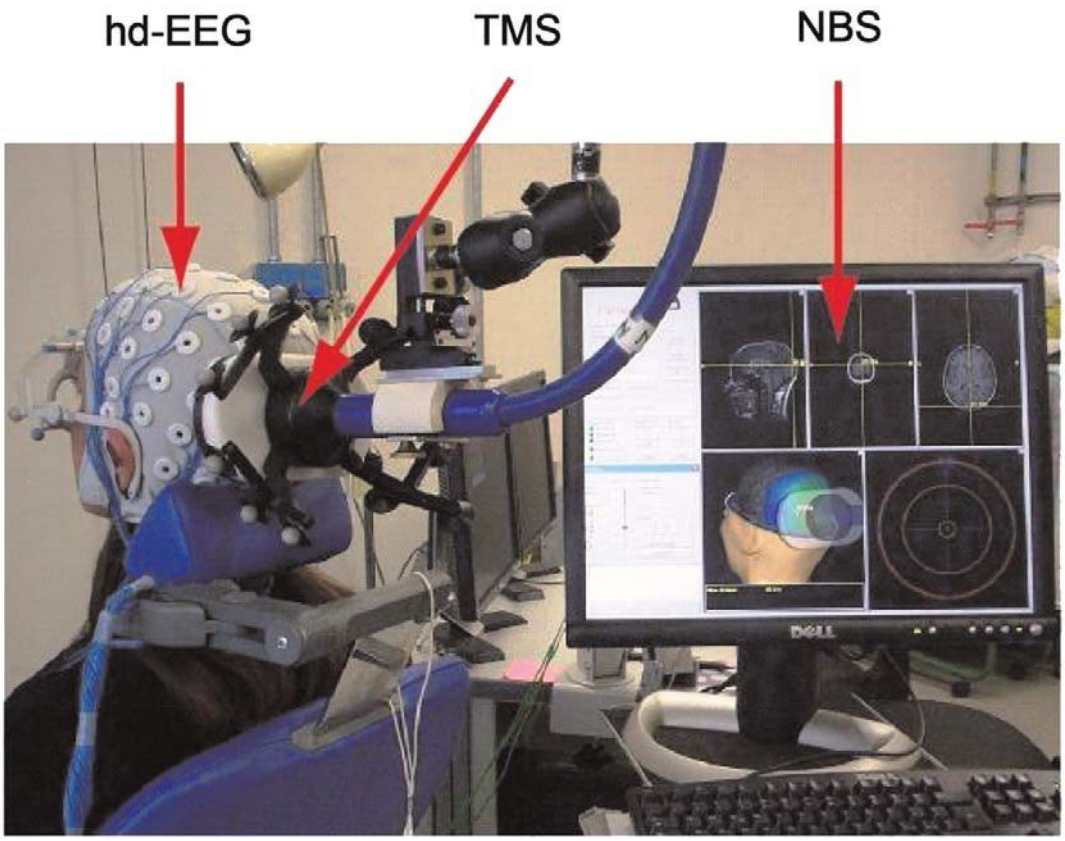

Figure 4.1 A classical TMS-EEG setup combined with navigated brain stimulation system (NBS) (adapted from [3]).

The combination of TMS with EEG allows the recording of the effects of the perturbation on both the stimulated area and on other distant cortical areas, just a few milliseconds after the pulse. The rapid change in the magnetic field, generated by the TMs pulse, determines the activation of the neural populations below the coil. The synchronized volley of action potentials thus generated diffuses along the available connection pathways, and can produce relevant electrical activations in the target and in interconnected brain regions. This synchronized activity can be recorded by the EEG, which offers then a readout of the effects of the stimulation on brain activity.

4.2 The variability problem

Thanks to its ability to directly influence brain activity, and promptly assess the effects of such influence, TMS-EEG has been widely used, across the years, both for research and clinical purposes [5,6]. However, one of the main problems associated with TMS is its high degree of interindividual variability: it concerns both behavioral and electrophysiological outcome measures (such as motor-evoked

potentials, MEPs, and TEPs) [7,8] specific stimulation protocols [9], limiting the efficacy of this technique in both research and clinical applications [6,8,9].
It is therefore fundamental to start thinking of shared practices in the world of TMS, to allow the birth of standardized procedures in TMS experiments which could increase the experimental control on confounding variables and reduce the amount of variability in the obtained results [10].

4.3 Aim of Experiment 3

The aim of the following experiment is to shed light on the influence of different stimulation parameters, such as coil position and coil orientation, on the quality of TEPs. By testing different coil positions and orientations, we aim at clarifying the role of these two factors in the choice of a "good" hotspot for TMS stimulation, in order to increase reproducibility in TMS results coming from different research groups and clinicians.

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5. EXPERIMENT 3

5.1 Introduction

Transcranial magnetic stimulation (TMS) is one of the most popular non-invasive brain stimulation techniques in the field of cognitive neuroscience, thanks to its capacity of inserting predefined quantities of energy into specific cortical areas, thus modifying neural activity and the associated cognitive processes and behavior [1]. When a brief and rapidly changing current pulse passes through a TMS coil, it generates a powerful magnetic pulse, capable of penetrating scalp and skull, and reaching the targeted brain area with minimal tissue attenuation. This magnetic pulse depolarizes the membrane of neurons located beneath the coil, giving rise to action potentials and resulting in neurophysiological and/or behavioural effects depending on the stimulated area [2].

TMS is often paired with brain mapping technology, such as EEG. This combination allows to visualize which areas in the brain are influenced by TMS, shedding light on spatiotemporal network dynamics and on the role of the different areas involved in a specific cognitive task [3]. TMS-EEG allow the recording of cerebral responses to the magnetic pulse. In this way, TEPs can be recorded with a temporal resolution in the order of milliseconds, which is an appropriate time scale to study neuronal responses from the brain [3]: therefore, TMS-EEG offers an excellent temporal resolution for studying brain dynamics [4].

Unfortunately, the efficacy of TMS-EEG is limited by high inter-subject and intra-subject variability, both in research and clinical applications [2,5]. This has hindered the reliability of TMS application, either preventing its diffusion as a clinical treatment for neurological and psychiatric conditions [6,7], or limiting the reproducibility of results obtained in TMS-EEG experiments [8]. This may be due to a number of reasons, both internal and external to the participants [9]. While the first ones are harder to control for, the latter are in principle easier to manage, since they mostly consist in the TMS parameters chosen to stimulate. One example of such external factors is the TMS hotspot, which is the coil position and orientation that, when stimulated, elicits the strongest and most reliable effect. A properly optimized hotspot search procedure should be able to control for the variability associated with where and how the stimulation coil should be positioned exactly.

However, at the moment there are no guidelines offering a standardized procedure for optimal hotspot search.

The objective of this study is therefore to shed some light on the influence that coil position and orientation have on determining a good TMS hotspot, trying to disentangle the contribution of these stimulation parameters in determining a good quality brain response.

This would constitute a first step towards an automated procedure for hotspot detection, contributing to a standardization of TMS-EEG studies that would reduce inter- and intra-subject variability and increase reproducibility between different subjects and experimental protocols, allowing for more direct comparisons between results of different research groups and clinicians [10].

5.2 Materials and methods

Participants

The study population included eleven right-handed participants (7 F, mean age 27±7.73). Exclusion criteria included having a history of either strokes, seizures, major neurological and psychological disorders or of significant head injury or trauma. Furthermore, a family history of epilepsy, medical implants, and pieces of metal in the head, regular drug use and pregnancy were also considered as exclusion criteria. Lastly, any other contra-indication for MRI or TMS excluded a participant from the study. In accordance to the 2013 Declaration of Helskink, we obtained from the participants written informed consent. The experimental protocol was approved by the local Ethics Committee.

MRI image acquisition

Participants underwent MRI with a 3 Tesla Siemens PRISMA scan to acquire anatomical T1-weighted images (TR 2.7 ms/TE 3.68). The acquired image was later used to neuronavigate the TMS coil.

TMS-EEG setup

For the experiment we used TMS-compatible EEG equipment to register EEG and EMG activity (NeurOne Tesla, Biuttium Biosignals Ltd., Finland), combined with a Fast'n East cap with 64 TMS-compatible Ag/AgCl pellet pin electrodes (EasyCap

GmbH, Herrsching, Germany) placed following the extended 10-20 International System. Online reference was placed over FCz, while the ground electrode was on POz. Electrode impedance was kept below 5 KΩ. Biphasic single-pulses were delivered with a focal TMS coil (C-B60, MagVenture) which was held by a mechanical arm and connected to a TMS device (MagPro X100 with MagOption, MagVenture). Furthermore, neuronavigation via an MRI-based frameless stereotactic system (TMS-Navigator, Localite) using individual anatomical MR images was employed.

Experimental procedure

During the experimental session, participants were seated in a reclining chair with their arms relaxed and their head stabilized with a vacuum cushion. The first step consisted of determining the motor hotspot for each participant, i.e. the specific region of M1 eliciting the largest and most consistent motor evoked potentials (MEPs) measured using EMG. EMG electrodes were placed on the first dorsal interosseous (FDI), the abductor pollicis brevis (APB) and the abductor digiti minimi (ADM) muscles of the participants' right hand. The TMS coil was then moved around the precentral cortical gyrus known to possess motor hand functions (the so called "hand knob") until a position that reliably elicited consistent MEPs was found. After that, TMS stimulation intensity was set individually for each participant by determining their resting motor threshold (RMT), which was later used as stimulation intensity. The RMT was defined as the lowest possible stimulation intensity sufficient to generate consistent MEPs (at least 50% of the TMS pulses resulting in a MEP amplitude of at least 0.05 mV) [11]. To determine the RMT, we employed the automatic procedure included in the BEST Toolbox [12] for MATLAB (Mathworks, USA). The starting intensity was set based on the intensities that elicited distinct MEPs during the motor hotspot search and the target EMG channel was selected according to which muscle responded most reliably during the motor hotspot search. Operating in a closed-loop circuit, the BEST Toolbox sets the intensity for each stimulation based on the EMG data acquired after the preceding stimulation. Within 40 trials, the protocol drove the intensity up and down based on the recorded MEP sizes to determine the minimum stimulus

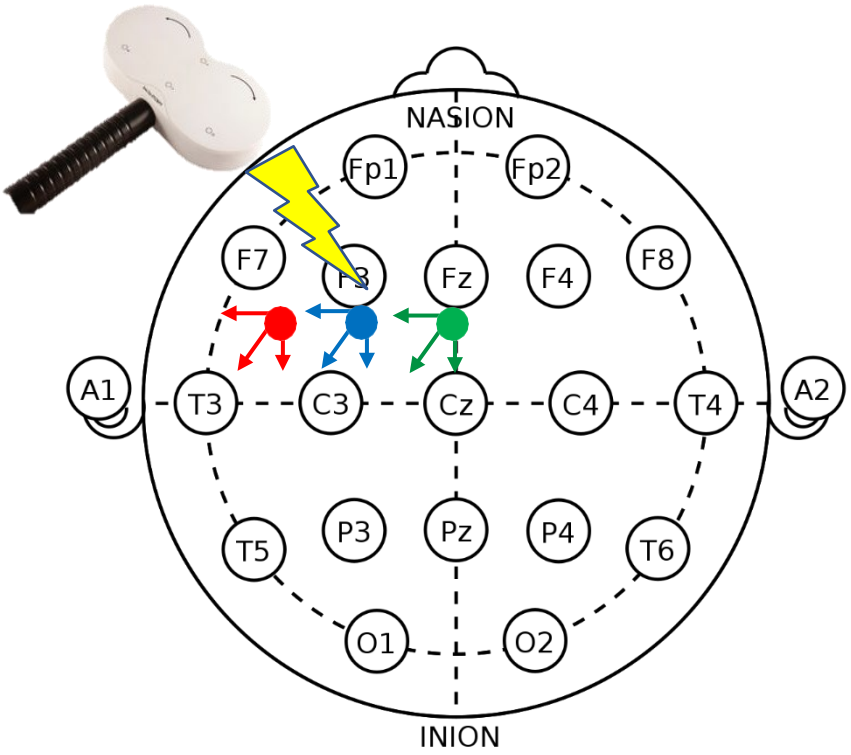

Figure 5.1 TMS was administered on three different positions (midline, superior frontal gyrus, superior frontal sulcus), each of them stimulated at three different angles (0°, 45°,90°).

intensity needed to generate consistent MEP. Once the stimulation intensity was established, the TMS targets were detected on the basis of individual MRIs. TMS was administered on three different areas: on the superior frontal gyrus (SFG) in correspondence of BA6, medially on the brain midline and laterally on the superior frontal sulcus (SFS). For each of these targets, three different coil orientations were employed: 0°, 45° and 90° with respect to the rostral-caudal axis. It was ensured that no target nor orientation was able to elicit MEPs in the participant, in order to avoid somatosensory feedback confounds in the EEG. Once the targets were determined, the actual experimental session started. Participants wore earphones playing a masking sound created with the TAAC toolbox [13], which allows to create masking noises tuned to the specific auditory characteristics of the click of the TMS coil; the volume was adjusted to the participant's comfort limit. Participants were then asked to look at a fixation cross on a screen, to minimize distractions and eye movements. They underwent nine blocks of TMS stimulation, one for each combination of site (midline, SFG, SFS) and orientation (0°, 45° and 90°); the order of the stimulation conditions was randomized across subjects (Fig.

5.1). Each block comprised 150 pulses, administered randomly every 2 to 3 seconds. Blocks were interleaved with pauses to allow the participant to relax and reposition the coil.

EEG preprocessing

Data were analyzed with the MATLAB toolbox EEGLAB [14] and the TMS-EEG signal analyzer (TESA) extension [15]. First, the continuous raw signal digitized at 5000 Hz was segmented 800 ms before and after the TMS pulse. Then, the TMS pulse artifact was removed from -3 to 8 ms, and data were demeaned using the whole epoch. A manual trial-by-trial epoch rejection was then performed on each dataset, to check for artifacts that may hinder ICA decomposition [16]. A first round of ICA was then performed on each participant to remove the TMS artifact. A time window of 15 ms (from -5 to 10) was then removed around the TMS pulse from each session to account for the remaining TMS artifact and replaced with cubic interpolation to avoid the creation of ringing artifacts. Data were then downsampled at 1000 Hz, bandpass-filtered (1-80 Hz, zero-phase, fourth-order Butterworth band-pass) and band-stop filtered (49-51 Hz). The interpolated data around the TMS pulse were then removed and substituted with constant amplitude data, and a second run of ICA was used to check for blinks, lateral eye movements, persistent muscle activity, and electrode noise. Data around the pulse were then cubic interpolated again, and then average referenced. Finally, data were epoched from -100 to 350 ms, bad trials were automatically detected and rejected through the TBT toolbox [17] (extreme values thresholds: +/- 125 μV, improbability and kurtosis criteria for single channels: SD > 5, for global threshold: SD > 3, maximum slope allowed: 50 μV, and minimal R squared allowed: 0.3), and baseline corrected.

Statistics

A 3x3 2-way repeated measures ANOVA was performed on TEPs with *coil orientation* and *coil position* as within-subject factors. A temporal threshold was then applied to the data in order to maintain only those activations equal to or longer than 10 ms.

5.3 Results

In order to describe TEP results, we calculated GMFP for each different condition and used the peaks to determine different clusters.

Our preliminary two-way repeated measures ANOVA showed significant activations [all ps < .05] of *coil orientation*. We detected three main clusters: the first one in the 34-61 ms time window, comprising electrodes FCz, C4, C2, C5, CPz, TP9, involved mainly bilateral central electrodes; the second one, in the 131-254 ms time window, comprising electrodes Fp1, AF8, AFz, AF7, F10, F8, F6, F2, Fz, F1, F3, F9, FC6, FCz, FC5, FT9, T8, C4, C2, Cz, C1, C5, T7, CP6, CP4, CP1, CP3, TP9, P8, P6, P4, P2, P5, PO8, PO7, involved mainly bilateral frontal, central and parietal electrodes, being spread over the whole scalp; the last one, in the 254-350 ms time window, comprising electrodes FP2, AFz, Fz, F7, FT10, C6, C3, CPz, CP5, P8, P3, PO3, involved mainly bilateral frontal, central and parietal electrodes (Fig. 5.2A).

Significant activations were found also for the *coil position*. A first cluster was found in the 75-132 ms time window, comprising electrodes Fp1, AF7, F6, F3, F5, FC4, FCz, T8, C4, C1, C5, TP10, TP8, TP9, P6, PO4, PO7, and involved mainly bilateral fronto-central and temporo-parietal electrodes; a second was present in the 254-350 ms time window, comprising electrodes AF8, AF4, F4, FC6, FC4, FC1, FC3, FT9, T8, Cz, CP6, CP4, CP3, TP7, P2, PO8, O2, O1, and involved mainly fronto-central, centro-parietal and parieto-occipital electrodes (Fig. 5.2B).

Also in the interaction we found three clusters of significant activity. The first one, in the 73-130 ms time window, comprising electrodes AFz, AF7, F10, F9, FT10, FCz, Cz, CP5, P1, involved mainly left fronto-central electrodes; the second, in the 130-255 ms time window, comprising electrodes Fp1, F6, F9, FC4, FC5, FT7, P2, Pz, P7, involved mainly bilateral frontal and parietal electrodes; the third one, in the 255-350 ms time window, comprising electrodes Fp2, FpZ, AFz, AF7, Fz, F1, F3, F9, FT10, FT8, FC6, FC4, FC2, FC3, FC5, FT7, C6, C4, C2, C1, C5, CP2, CPz, CP5, TP7, TP9, P6, Pz, P5, P7, PO7, PO3, was bilaterally widespread over the whole scalp (Fig. 5.2C).

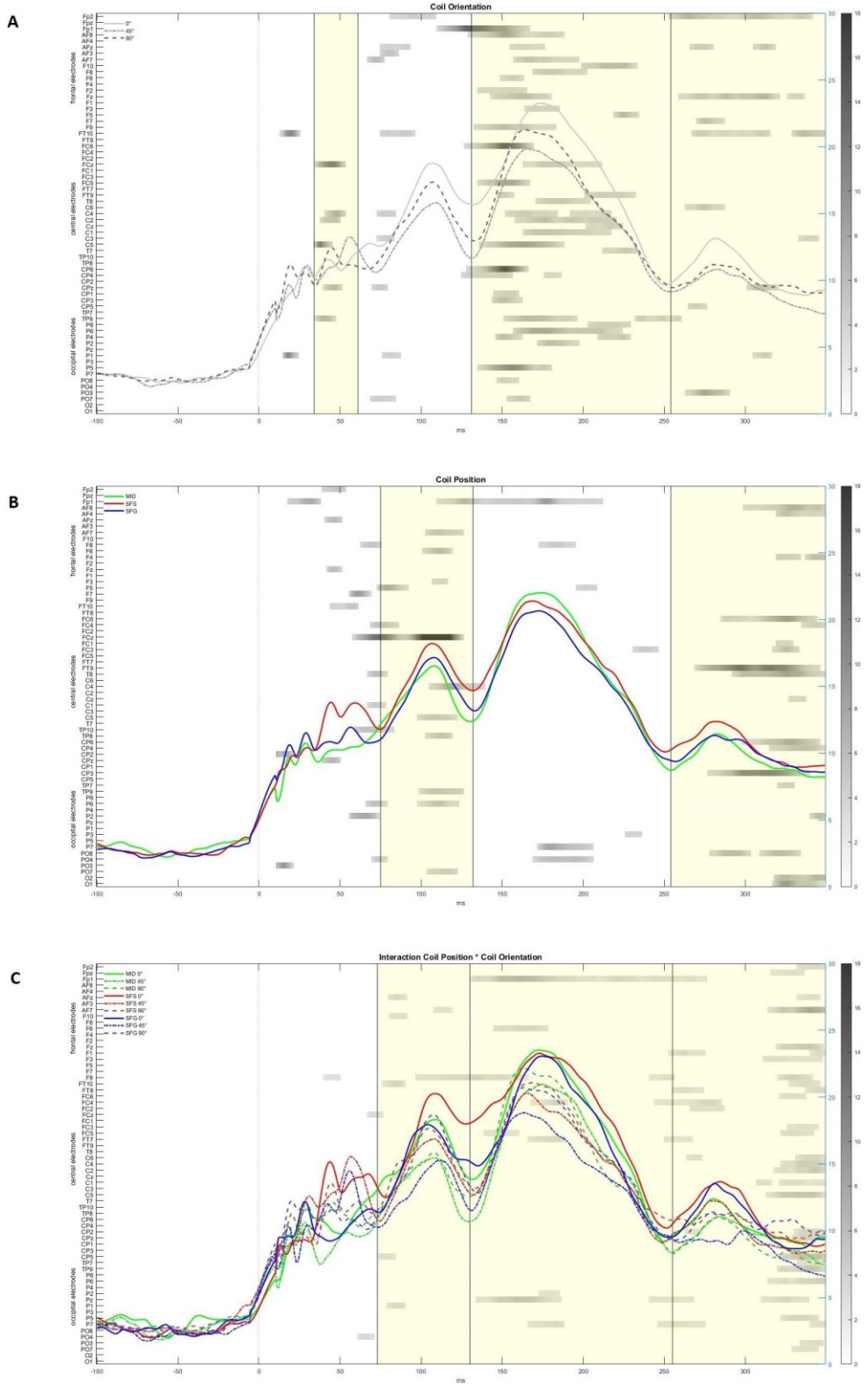

Figure 5.2 Results from the ANOVA conducted on TEPs: main effects of "Coil orientation", "Coil position" and their interaction. **A**: Raster plot depicting for each time point and each electrode the significant differences (expressed in F-values) between the three stimulation angles. X and Y axis respectively represent time in milliseconds and electrodes (from posterior to anterior ones). The lines superimposed represent the associated GMFPs (see right Y axis, expressed in μV), whose peaks were used to identify TEPs clusters. **B**: Raster plot depicting for each time point and each electrode the significant differences between the three stimulated positions. **C**: Raster plot depicting for each time point and each electrode the significant differences between the nine conditions in the interaction.

5.4 Discussion

The aim of this experiment was to try to ascertain the influence that different stimulation parameters – specifically the coil position and orientation – can have on the elicited TMS activity. In this way we tried to define the features that characterize a TMS "hotspot", i.e. a TMS target able to determine a good brain response, from a "coldspot", which can only elicit a suboptimal brain response. To achieve this, for each participant we administered TMS on three different positions – superior frontal sulcus, superior frontal gyrus, and midline, all of them in correspondence with Brodmann area 6 –, each of them with three different coil orientations – 0°, 45° and 90° – for a total of nine different combinations.

Our preliminary results seem to show the existence of differences in how the brain reacts to TMS, depending on both the stimulated site and the chosen orientation. Changing these parameters, in fact, affects the amplitude of the elicited response. While this is true for both factors, coil orientation is the one that seemingly has the stronger influence. This comes with little surprise: in fact, the TMS targets are all belonging to BA6, each of them being only a few centimeters apart, while it is known that the major differences in the elicited TEPs come from the stimulation of different brain areas [18,19].

On the other hand, coil orientation seems to have a stronger influence on the elicited brain response. A series of studies has tried to investigate the role of coil orientation on brain activity [19–21], disentangling the contribution of different stimulation angles on the desired outcome of measures, in particular on the evoked MEPs [22,23]. A further level of analysis has sometimes been considered, studying the correspondence between the electric field (E-field) induced in the brain tissue by the different stimulation angles, and the obtained brain response [24]. This approach, in addition with the possibility of considering the participant's individual variability in neuroanatomy, could allow a better individual optimization of the stimulating parameters.

Further analysis will go in the direction of better characterizing the brain response to variations in the parameters of stimulation, including E-field calculation for each combination of parameters.

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General conclusions

In the first part of this work, we have tried to investigate the founding mechanisms of visual awareness, with a particular focus on the existence of hemispheric asymmetries.

In two different TMS-EEG experiments we stimulated in both hemispheres the early visual cortex and the intraparietal sulcus, two areas known to give rise to visual percepts when stimulated. The results were interestingly matching: in fact, the patterns associated with phosphene perception were apparently more different across different hemispheres than across different areas: both left early visual cortex and left IPS, in fact, showed an early time window of differential activity correlating with visual perception; on the other side, both right early visual cortex and right IPS showed differential activations in a much later time window. These results suggests that, while early visual areas and IPS are two independent phosphene generators, they might share an intrahemispheric activity pattern, responsible for the commonalities we found.

In the second part, we considered the influence that various stimulation parameters can have on the TMS-evoked brain response. It is well known that the elicited brain activity can be influenced by a series of factors, some of them independent from the researcher, and others that can be more easily controlled. Amongst these latter, coil orientation and coil position are known to be able to influence the effect of stimulation. Our preliminary results show that variations in these parameters can significantly alter the brain response, underlining the necessity for a more thorough analysis capable of disentangling the actual contribution of these two parameters.