



TMS-EEG approach unveils brain mechanisms underlying conscious and unconscious face perception



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ABSTRACT

Background: Conscious perception of external stimuli has been related to recurrent activity in distributed cortical networks, although brain mechanisms controlling unconscious processing and stimuli access to conscious report need to be clarified.

Objective: This study aims at investigating modulations in cortical excitability related to conscious perception and unconscious processing of face stimuli with different visibility levels.

Methods: We used TMS-EEG over the right occipital face area (rOFA), or the right premotor cortex (rPMC) as control site, to measure cortical excitability during a backward masking paradigm with individually defined stimuli visibility.

Results: Event related potentials showed significant differences for faces compared to houses, and detected faces compared to missed ones, 200 ms post target onset. TMS over rOFA, but not over rPMC, triggered a relative positivity starting 150 ms post target when faces with high visibility were consciously reported. Moreover, rOFA TMS evoked differential responses for high versus low visible faces in conscious and unconscious processing at 290–390 and 180–240 ms, respectively.

Conclusion: Results unveiled a causal link between rOFA excitability and late responses related to access to conscious perception, suggesting a critical role of recurrent activity, but distinct components, for consciously perceived stimuli and unconscious face processing.

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Introduction

The neural mechanisms related to conscious perception, considered as the access to the conscious report of an external stimulus [1], represent a challenging research field in neuroscience. Up to now, both feed-forward and top-down models [2,3] have been proposed to explain brain correlates of perceptual awareness, supported by data showing electrophysiological correlates of conscious report either in early occipital component, or in late component localized in fronto-parieto-temporal network. Recently, the Global Neuronal Workspace model (GNW) [4–6] posited that access to consciousness is related to distributed and long-range

cortical connections between thalamo-cortical loops and neurons in prefrontal, cingulate and parietal regions, which amplify and sustain stimuli representation. Posterior specialized areas, e.g. extra-striate cortices for object, face or motion perception, contribute with bottom-up and recurrent projections, but only stimuli encompassing specialized processing eliciting a threshold level of activity in the prefrontal cortex get access to consciousness.

Several studies investigating the neural correlates of conscious and unconscious visual perception employed faces as stimuli in different experimental paradigms. This category of visual stimuli is highly relevant in the human environment, conveying multiple information and requiring the coordinate activity of different cognitive mechanisms, underpinned by distributed brain circuits, to be processed [7,8]. Converging evidence have suggested the role of a cortico-subcortical network, encompassing the amygdala, insula, cingulate cortex, hippocampus and fusiform gyrus, in unconscious processing of emotional and social features of faces [9].

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Conversely, the neural correlates for conscious and unconscious processing of non-emotional faces remain unclear, as well as the mechanisms leading uncertain stimuli to cross the perceptual threshold in order to be consciously reported. In electrophysiological studies, the N170 component is reported as a well-established correlate of conscious face perception [10]. Previous studies reported that the N170 was abolished [11] or did not differentiate face from non-face stimuli [12–14] when faces were processed in absence of awareness, supporting a relationship between limited access to consciousness and disruption of feedforward mechanisms. On the other hand, modulations of the N170 for stimuli non-consciously reported suggested the encoding of visual features in early electrophysiological correlates even in the absence of perceptual awareness [15–17]; in this case the integrated activity between different areas of the network may represent the critical step for access to conscious report [18].

The present study aims at clarifying the temporal and spatial distribution of brain mechanisms involved in conscious and unconscious face processing with a novel paradigm, combining Transcranial Magnetic Stimulation with EEG recordings (TMS-EEG). Critically, this approach allows assessing not only the ongoing response of the cerebral cortex to the visual input recorded by the ERPs, but also the TMS-evoked potentials (TEPs), which are considered a reliable measure of cortical excitability, unveiling the functional status of the area and its connectivity with the active network in specific time frames [19]. A backward masking paradigm was used, individually defining different visibility thresholds by adding visual noise to the presented stimuli; during the task, the right occipital face area (rOFA) [20] was targeted, with a single TMS pulse at 60 ms from stimulus onset, and EEG was continuously recorded with a 60-channel TMS compatible amplifier. The timing of TMS pulse was defined on the basis of previous studies showing that the rOFA contributes to face discrimination at this early stage of stimulus processing [20,21]. The backward masking paradigm allowed to compare responses for different types of stimuli (face vs control stimuli, i.e. houses) in different visibility conditions and for stimuli with identical visual features, but when correctly reported or missed by participants. In particular, we aimed at measuring the timing and cortical distribution of brain excitability induced by rOFA stimulation, testing whether responsiveness in the face processing network was modulated by the detection of faces at different visibility levels and by face processing in conscious and unconscious conditions. In order to test the site specificity of the effects related to rOFA stimulation, the right premotor cortex (rPMC) was stimulated in a control experiment; since this area is not expected to have a role in face processing [22] cortical activity induced by rPMC TMS should not be affected by the different experimental conditions. Differently, since rOFA is known to be involved in early stage of face processing [20], we hypothesized that face visibility would affect cortical excitability probed by TMS, and we predicted different responsiveness for correctly detected faces as compared to non-face stimuli and missed faces. Moreover, differential TMS-responses related to face visibility for missed faces would suggest an involvement of rOFA in processing unconscious stimuli.

Material and methods

Participants

Fourteen healthy volunteers (2 males, mean age 24.78, SD 3.9 years) participated in the study. One participant was excluded from the analyses because of a high number of trials rejected due to low performance in the experimental task and noise in the EEG recording. The study took place in the TMS-EEG laboratory of the

University of Milano-Bicocca with the approval of the local Ethic Committee and all participants signed written informed consent prior to their participation.

Procedure

Stimuli consisted of 14 faces (7 males, 7 females) with neutral expression from the Karolinska Directed Emotional Faces set (KDEF) [23] and 14 houses selected from a free set (<http://agingmind.utdallas.edu/other-stimulus/>). All images were converted in grey scale, resized to 565 × 703 pixels and mean brightness was normalized across the images. Stimuli were then encircled in a grey contour oval shape to remove background and contextual differences. A grey scale mask image was created by scrambling random part of all the images. At the beginning of the experimental session each participant completed a QUEST adaptive stair-case procedure [24] to define individual thresholds for face detection. The QUEST procedure was implemented by using the Psychophysics Toolbox Version 3.1.11 (PTB-3) [25,26] within Matlab 2013 and consisted of 140 trials presenting 70 face and 70 house stimuli in random order. Each trial started with a fixation cross of 2000 ms, followed by a 34 ms target and the mask image for 516 ms; then the question “Have you seen a face?” appeared on the screen until the participants gave a response by pressing 1 or 2 keyboard buttons. The images were presented with added Gaussian noise, which could vary from 0 to 1, starting from 0.9 and then adjusted throughout the QUEST procedure according to the participants’ correct or incorrect responses. The β , δ and γ parameters of the Weibull psychometric function underlying the QUEST procedure were set to 3.5, 0.5, and 0.5, respectively. At the end of the QUEST procedure, noise levels in which the participant correctly detected 55% or 85% of faces were selected to create the stimuli for the low and high visibility condition, respectively. Thus, individually prepared stimuli were used for the main TMS-EEG experiment. This consisted in 6 blocks of 168 trials with faces or houses in low or high visibility condition presented in random order. Two blocks were completed with only EEG recordings (ERP blocks), whereas in four block a single pulse TMS was concurrently administered during the task (TEP blocks) at 60 ms from face onset over rOFA (see Fig. 1 for trial timeline). Experiments were run using E-prime software (Psychology Software Tools, Pittsburgh, PA), recording accuracy and reaction times (RTs). The order of blocks with and without TMS was counterbalanced across participants.

TMS stimulation

TMS was delivered with an Eximia TMS stimulator (Nexstim, Helsinki, Finland) using a focal bi-pulse, figure of eight 70-mm coil. The rOFA was selected as target site using the Talairach coordinates $X = 38$ $Y = -80$ $Z = -7$ [20]. Individual MRIs were normalized on standard template and the transformation matrices were used to convert Talairach coordinates in individual coordinates for each participant MRI structural space. The target site was then reported in the Navigated Brain Stimulation system (NBS, Nexstim, Helsinki, Finland), which uses infrared-based frameless stereotaxy to map the position of the coil and the subject’s head within the reference space of the individual’s MRI space. The NBS system also estimates the electrical field induced by TMS taking into account coil position and orientation, distance from scalp and head shape. According to the NBS system TMS was delivered at an estimated mean intensity of 89.37 ± 10.12 V/m ($59.65 \pm 3.73\%$ of the stimulator output). As in previous studies [22,27–29], a masking noise was continuously played into earplugs worn by the participants during the experimental sessions in order to avoid auditory EEG responses evoked by the TMS coil discharge.

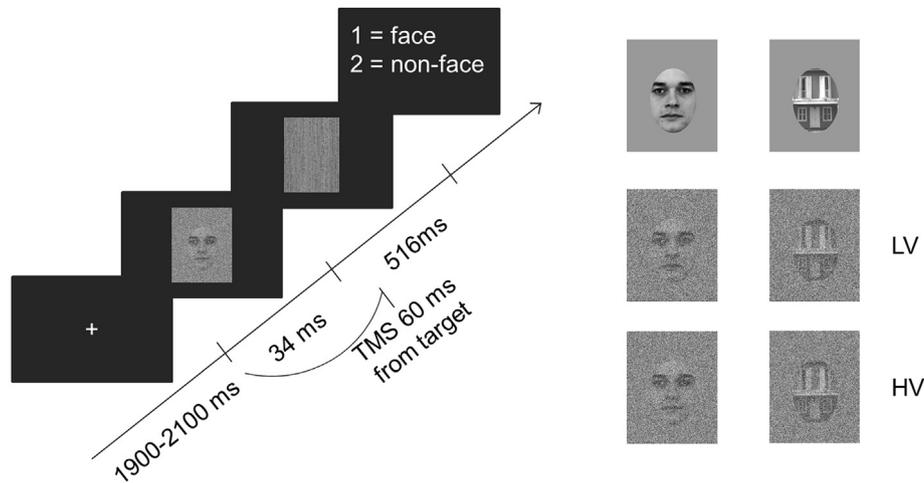


Fig. 1. Time-line of one trial in the backward masking task and example of stimuli. Stimuli appeared for 34 ms followed by 516 ms mask; the participants were then asked to report whether they detected a face or not by pressing two buttons with the right hand. Trials were interleaved by a fixation cross which remained on the screen for jittering time between 1900 and 2100 ms. In TEP blocks a single pulse TMS was administered at 60 ms from face onset over rOFA, or rPMC in the control experiment. On left side of the figure there are examples of stimuli as originally created (top line) and with added Gaussian noise to create low (LV; middle line) and high visibility (HV; bottom line) condition.

EEG recording and analyses

EEG was recorded with a 60-channel TMS compatible amplifier (Nexstim; Helsinki, Finland) as described before [28,30]. Matlab R2012a (Mathworks, Natick, MA, USA) was used for pre-processing. Data were down-sampled to 725 Hz, continuous signal was split in trials between -800 and $+800$ ms from TMS pulse and the corresponding time-window for ERP blocks; trials with excessive artefacts were removed by a semi-automatic procedure [31] and signal was band-pass filtered between 1 and 45 Hz. Bad channels were interpolated using spherical spline interpolation function of EEGLAB [32]. TEPs were then averaged referenced and baseline corrected between -300 and -80 ms before the onset of the visual stimuli. Independent component analysis (ICA) was applied in order to remove residual muscular and magnetic artefacts. Trials were then divided on the basis of stimulus type (faces or houses), response accuracy (correct or incorrect target detection) and noise level (low and high visibility). Thus, TEPs and ERPs were computed by averaging selected artefact-free single epochs for each condition (see Supplementary Table 1 for number of accepted trials in each condition).

Cortical responses in different conditions were compared through a cluster-based test [33] implemented in the FieldTrip MATLAB toolbox for M/EEG analysis (freely available at <http://fieldtrip.fcdonders.nl/>) [34]. ERPs and TEPs blocks were separately analysed with whole-head, cluster-based permutation *t*-test. This procedure corrects for multiple comparisons by permuting the data and clustering them based on their spatial and temporal proximity. Conditions of interest were compared as follows: (i) effect of stimulus type only in correct responses: faces vs houses in low visibility condition, faces vs houses in high visibility condition; (ii) effect of accuracy in face detection: detected vs missed faces in low visibility condition, detected vs missed faces in high visibility condition; (iii) effect of visibility in conscious and unconscious face perception: high vs low visibility condition of detected faces, high vs low visibility condition of missed faces. For each comparison 10000 permutation were performed with a permutation-significant level of $p = .05$ for the time-window between 0 and 410 ms from target onset corresponding to -60 and 350 ms from TMS pulse in the TEP blocks.

Results

Behavioural

The QUEST resulted in a mean Gaussian noise of 0.6 (SD = 0.23) for the low visibility condition and 0.49 (SD = 0.22) for the high visibility condition. These two noise levels were significantly different between conditions [$t(12) = 9.94, p < .001$]. Mean accuracy scores in the behavioural task during the TMS-EEG experiment are reported in Table 1. ANOVAs with TMS (TMS vs no TMS), stimulus type (faces vs houses) and visibility condition (low vs high) as within subject factors were carried out on accuracy scores and reaction times for correct responses (RTs). For accuracy scores the main effects of stimulus type [$F(1,12) = 125.29, p < .001$] and visibility condition [$F(1,12) = 51.16, p < .001$], as well as their interaction [$F(1,12) = 30.81, p < .001$] were significant, whereas the main effect of TMS and other interactions were not significant ($ps > .15$). Accuracy was overall lower for face stimuli than non-target stimuli (houses), and, of course, greater for high visibility than low visibility condition. Post hoc tests Bonferroni corrected for the significant interaction showed that accuracy scores differed between visibility conditions for face stimuli ($p < .001$), but not for house stimuli ($p = .84$). The analyses on RTs revealed a significant main effect of stimulus type [$F(1,12) = 6.3, p = .027$], being participants faster in detecting faces ($M = 275.6$ ms SD = 86.9) than houses ($M = 342.5$, SD = 184). No other effects were significant (all $ps > .18$).

ERP

Electrophysiological results are summarised in Table 2, Figs. 2 and 3. As shown in the butterfly plots, the shape and amplitude of peaks from target onset to ~ 200 ms are similar across conditions, with signal peaking at ~ 80 and ~ 130 ms. Confirming this observation, cluster-based analyses testing the effect of stimulus type on correct responses and the effect of accuracy in face detection revealed significant positive cluster in anterior electrodes and negative clusters in posterior electrodes starting at 190 ms post target onset. The analyses testing the effect of visibility level in conscious (i.e. correctly detected target) and unconscious (i.e. missed target) face perception were not significant in ERP blocks (all $ps > .05$).

Table 1
Behavioural results.

Faces TMS		Houses TMS		Faces no TMS		Houses no TMS	
LV	HV	LV	HV	LV	HV	LV	HV
0.36 (0.13)	0.61 (0.21)	0.85 (0.17)	0.85 (0.15)	0.39 (0.14)	0.62 (0.19)	0.87 (0.13)	0.87 (0.14)

Mean accuracy (standard deviations in brackets) in the TMS-EGG experiment. LV = low visibility condition; HV = high visibility condition.

Table 2
Summary of time-range for significant clusters in electrophysiological analyses of rOFA TMS experiment.

ERPs	Effect of stimulus type: Faces vs Houses	
	LV	HV
	positive 220–280 ms; $p = .037$	positive 190–395; $p < .001$
	negative 195–280 ms; $p = .008$	negative 190–380; $p < .001$
	Effect of accuracy: Detected vs Missed Faces	
	LV	HV
	positive 210–320 ms; $p < .001$	positive 210–330 ms; $p = .003$
	negative 200–340 ms; $p < .001$	negative 200–350 ms; $p = .003$
TEPs	Effect of stimulus type Faces vs Houses	
	LV	HV
	positive 200–380 ms; $p < .001$	positive 190–380 ms; $p < .001$
	negative 195–380 ms; $p < .001$	negative 190–390 ms; $p < .001$
	Effect of accuracy: Detected vs Missed Faces	
	LV	HV
	positive 200–360 ms; $p < .001$	positive 150–410 ms; $p < .001$
	negative 210–350 ms; $p < .001$	negative 220–410 ms; $p < .001$
	HV vs LV	
	Conscious	Unconscious
	negative 295–390 ms; $p = .039$	positive 180–240 ms; $p = .029$

All times are expressed in milliseconds from the onset of visual stimuli. LV = low visibility condition; HV = high visibility condition.

TEP

A summary of significant clusters is reported in Table 2 and average butterfly plots for the different conditions are depicted in Figs. 4–6. TMS applied 60 ms post target onset produced a perturbation lasting until ~100 ms from target onset, then the EEG signal shows three peaks at 120–150 ms, ~230 ms and ~330 ms from target onset; the two latter peaks appeared greater and better defined in the TEP than in the ERP blocks. Cluster based analysis testing the effect of stimulus type showed that in both visibility conditions faces produced larger positivity in anterior fronto-central electrodes and larger negativity in posterior occipito-parietal electrodes with signal peaking at ~230 ms and ~330 from target onset (Fig. 4). The analysis on the accuracy effect in face detection for low visible stimuli showed significant clusters in a similar time range (Fig. 5 left panel). Differently, in the high visibility condition (Fig. 5 right panel), an earlier positive cluster was significant starting at 150 ms from target onset (corresponding to 90 ms following TMS pulse). Scalp topographies showed that this positive cluster started in posterior temporo-parietal right lateralized electrodes (PO4, P4, P8, CP6, TP8, TP10, T4, FT8, FT10) located near the TMS target, moving then to frontal electrodes.

Finally, cluster-based analyses testing the effect of noise level for detected faces (i.e. conscious perception) revealed a significant negative cluster including posterior parietal electrodes and peaking at ~330 ms. Whereas for missed faces (i.e. unconscious perception) a positive cluster, including central electrodes and peaking at ~230 ms, was significant (Fig. 6).

Control experiment

In order to exclude that our results were simply due to unspecific TMS effects interfering with the EEG recording, a control

experiment was carried out, stimulating the rPMC, as a control area. Twelve participants (3 male, mean age 27.17, SD 4.39 years) were submitted to the same experimental procedure as above with TMS applied to the rPMC (Talairach coordinates $X = 19, Y = 5, Z = 72$; [22]) at the estimated mean intensity of 98.5 ± 7.55 V/m ($56.33 \pm 3.91\%$ of the stimulator output). As in the main experiment, the QUEST procedure, used to individually define stimuli visibility, resulted in a mean Gaussian noise of 0.64 (SD = 0.25) for the low visibility and 0.49 (SD = 0.24) for the high visibility conditions respectively [$t(11) = 4.87, p < .001$]. Data were pre-processed and analysed as above (see Supplementary Table 2 for number of accepted trials in each condition). Electrophysiological analyses on ERP blocks confirmed the presence of significant differences between stimulus types and accuracy in face detection starting at ~200 ms from target onset (see Supplementary Table 3 for summary of significant clusters). TMS applied over the rPMC produced a large cortical response peaking at 75 ms from target, corresponding to 15 ms from TMS pulse (Supplementary Figs. 1 and 2). Cluster-based analyses testing the effect of stimulus type (i.e. detected faces vs houses) in the low visibility condition showed a significant posterior negative cluster between 300 and 350 ms ($p = .043$) and a significant fronto-central positive cluster between 210 and 340 ms ($p < .001$) from target onset. Similarly, in the high visibility condition significant clusters were present at ~250 ms from target onset, with a topographical distribution highlighting an anterior positivity ($p = .003$) and a posterior negativity ($p < .001$). Analyses on accuracy effect in face detection (i.e. detected vs missed faces) for low visible stimuli showed two significant negative clusters in posterior electrodes (240–295 ms, $p = .042$; 300–345 ms, $p = .045$), whereas for high visible faces an anterior positive cluster and a posterior negative cluster were significant between 230 and 310 ms ($p < .001$) (Supplementary Table 3). Other contrasts between visibility conditions in detected and missed faces did not show significant results (all $ps > .05$).

Discussion

In the present study we measured brain excitability modulations induced by rOFA TMS related to face processing in conscious and unconscious conditions. The rPMC was stimulated as a control site to test for unspecific effects of TMS perturbation.

ERPs

Significant greater positivity in frontal electrodes and negativity in parieto-occipital electrodes starting from ~200 ms were present for correctly reported faces compared to houses (Fig. 2) and for detected faces compared to missed ones, both in low and high visibility conditions (Fig. 3). These results are consistent with previous findings showing modulation of early posterior negativity (EPN) and late positive potentials (LPP) during face processing [35,36]. Both these mid- and late-latency components have been reported as being modulated by emotional valence and arousal intensity of the stimuli, and by attention and awareness in visual processing [35,37,38]. Our data are consistent in terms of time-range and topographical distribution with a modulation of the

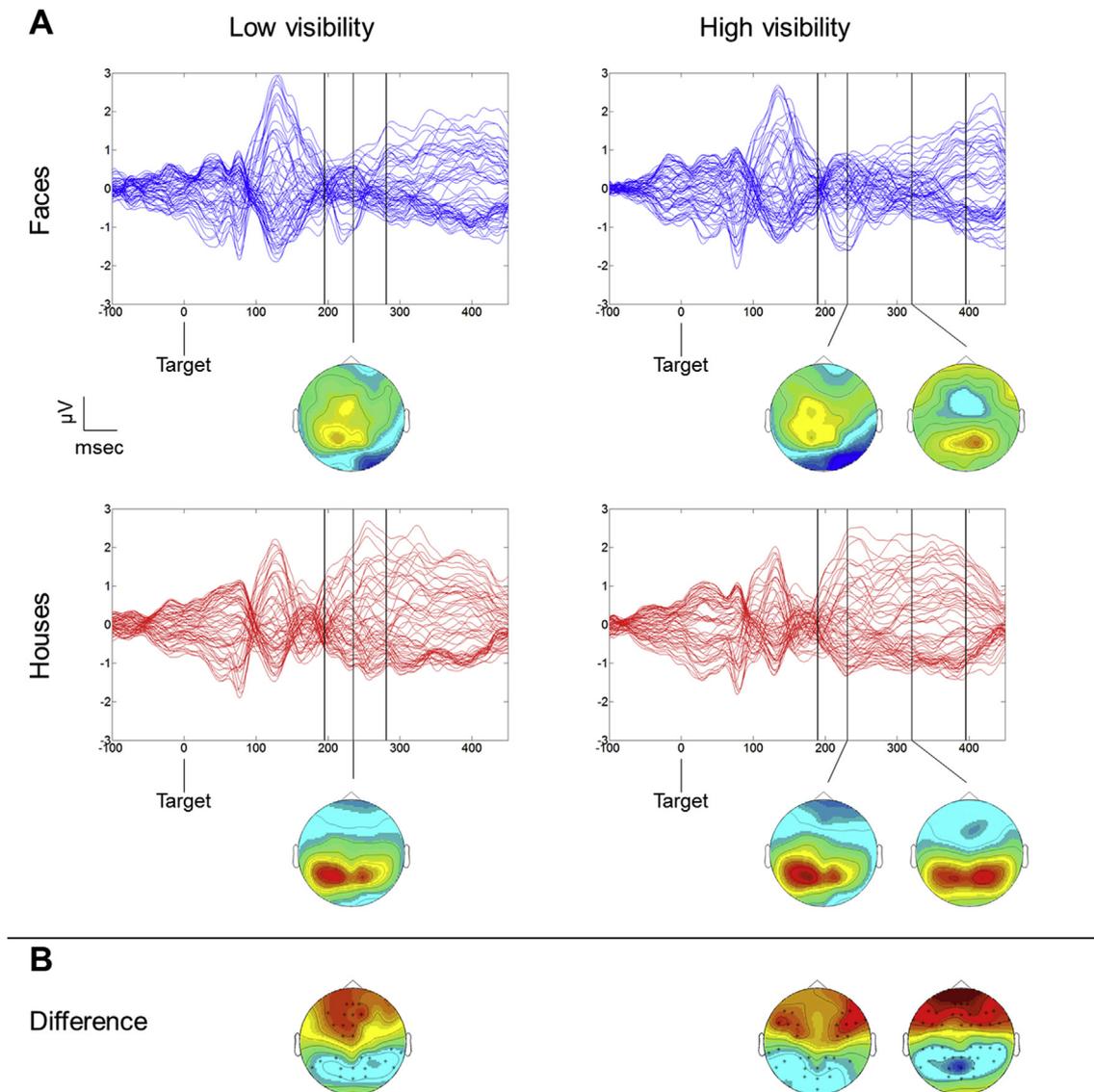


Fig. 2. ERP effects of stimulus type. Panel A: Butterfly plots of average ERPs for detected faces (blue) and houses (red) in low and high visibility condition; thick lines delimit the time-windows with significant differences between the faces and houses in the cluster-based analyses ($p < .05$, permutation corrected); thin lines indicate the timepoint corresponding to the topoplots of activity maps depicted below. Panel B: Topoplots of statistic maps for faces versus houses comparison; bold channels represent scalp location where differences were significant. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

EPN and LPP for comparison between face and non-face stimuli and between detected and missed faces, thus suggesting late mechanisms in conscious visual perception. In particular, differences between stimulus categories showed longer lasting effects in high than in low visibility condition, suggesting greater sustained activity, which involved both EPN and LPP components, for stimuli detected with higher level of accuracy, thus processed with greater subjective awareness [14,37]. On the other hand, peaks at ~ 80 and ~ 130 ms from visual stimuli were clearly present in the EEG signal (Figs. 2 and 3), but we did not find significant differences in this early activity for stimulus category or accuracy in face detection. Two main reasons may account for this result. First, we used cluster-based analyses to have robust, data-driven, statistics, testing differences in the whole post target time-window avoiding to focus the analysis on a priori selected temporal windows [33,39]. This differed from previous studies showing effects on N170 components with analyses restricted to shorter time-windows, in few occipito-temporal electrodes [15,40]. The second reason might be

related to the highly demanding task we used. We individually defined the stimuli with a QUEST procedure [24] and mean accuracy in the behavioural performance showed that participants detected $\sim 38\%$ and $\sim 60\%$ of the stimuli in the low and high visibility condition, respectively; visibility was thus modulated as expected, but part of the stimuli were effectively masked also in the high visibility condition. Our results, thus, replicate previous studies reporting the absence of differences between stimuli categories in early visual components [12,14] when perceptual awareness was limited with masking paradigms.

TEPs

In rOFA TMS blocks, late peaks at ~ 230 ms and ~ 330 ms from target onset appeared more defined than ERPs' components recorded in blocks without TMS, and were differently modulated across experimental conditions. These peaks showed the typical topographical distribution of EPN and LPP components [38]. As

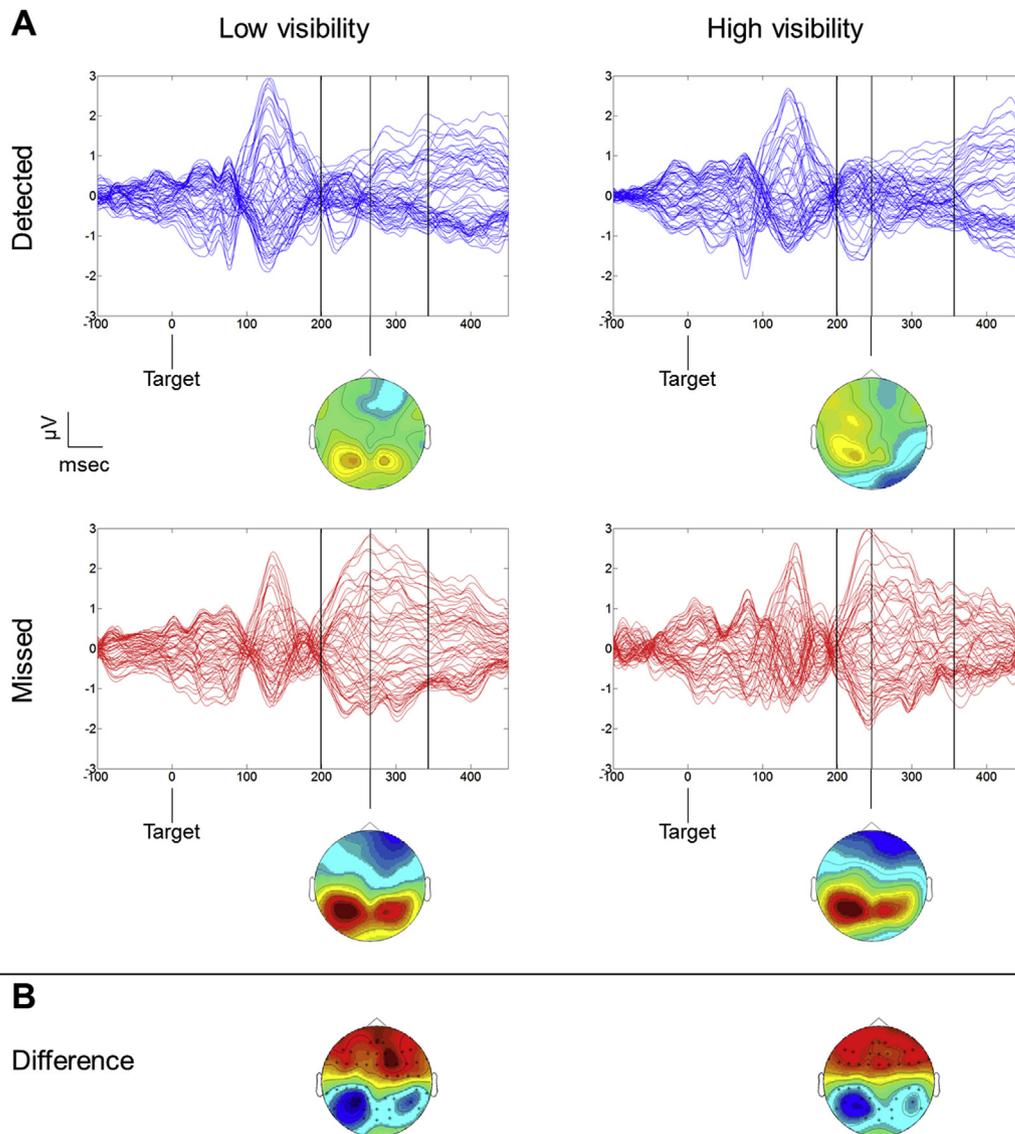


Fig. 3. ERP effects of accuracy in face detection. Panel A: Butterfly plots of average ERPs for detected faces (blue) and missed faces (red) in low and high visibility condition; thick lines delimit the time-windows with significant differences between detected and missed faces in the cluster-based analyses ($p < .05$, permutation corrected); thin lines indicate the timepoint corresponding to the topoplots of activity maps depicted below. Panel B: Topoplots of statistic maps for detected versus missed faces comparison; bold channels represent scalp location where differences were significant. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

predicted, TMS applied to rOFA during the face detection task highlighted differences in cortical excitability related to three main features: (i) the type of detected stimuli (face versus house), (ii) the access to conscious report (detected versus missed faces), and (iii) the level of visibility in both conscious and unconscious face processing. Differently, when TMS was applied over the rPMC differences between conditions were maintained in late components after 200 ms from the onset of visual stimuli, as appeared in the ERPs recording, but there were no further results for this control site.

Regarding the effect of stimulus type, target detection resulted in an increased modulation of the LPP for stimuli in low visibility condition when TMS was applied to rOFA, indicating a direct link between the activity of rOFA and responsiveness of the distributed centro-parietal network involved in this component. In particular, our data suggest that modulation of LPP may be related to the presence and the perceptual threshold of the critical stimulus: LPP, indeed, differentiated faces from houses when stimuli visibility was

set to make the target detectable above chance level in both ERP and TEP data. Conversely, while ERPs analysis did not show modulation of LPP for stimuli detected below chance level, rOFA TMS revealed that, even in such cases, a face-specific effect was present in LPP when cortical excitability was probed by TMS. Thus, by means of TMS-EEG recording, we demonstrated a causal relationship between the signal triggered in rOFA and late face-specific response related to face discrimination at different perceptual thresholds. Alternatively, the LLP modulation might depend more on the task request than the stimulus type. Participants, indeed, were explicitly asked to detect faces (and not houses), therefore, the observed cortical response might have been triggered by attentional resource towards target [41], regardless of being faces or houses.

Results concerning the accuracy effect in face detection support the role of EPN and LPP components in accessing visual consciousness. Peaks at ~230 ms and ~330 from target onset, indeed, were modulated by participants' subjective reports, with higher

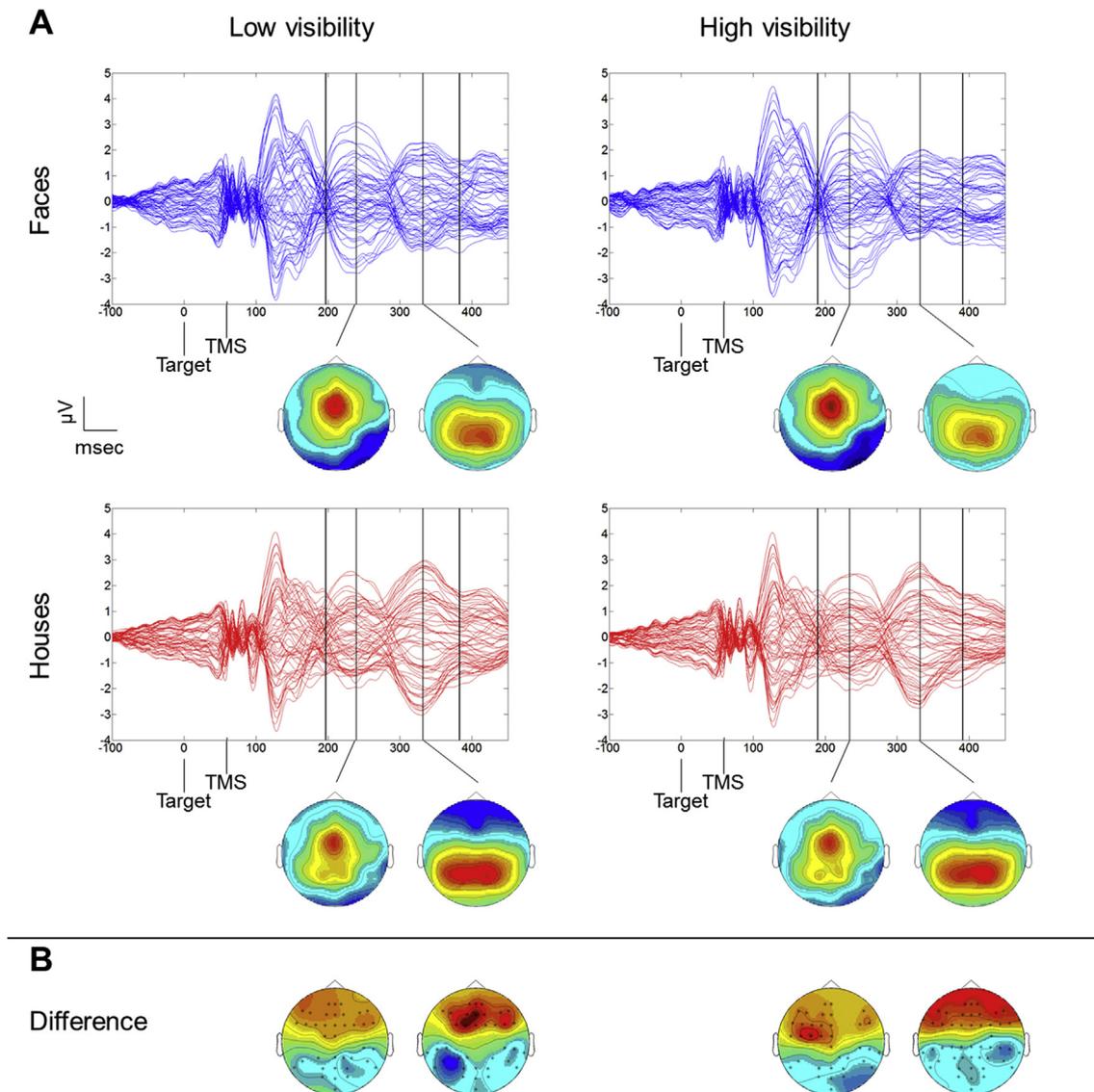


Fig. 4. TEP effects of stimulus type following rOFA stimulation. Panel A: Butterfly plots of average TEPs for detected faces (blue) and houses (red) in low and high visibility condition; thick lines delimit the time-windows with significant differences between faces and houses in the cluster-based analyses ($p < .05$, permutation corrected); thin lines indicate the timepoint corresponding to the topoplots of activity maps depicted below. Panel B: Topoplots of statistic maps for faces versus houses comparison; bold channels represent scalp location where differences were significant. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

posterior-negativity and anterior-positivity for detected compared to missed faces, both in low and high visibility conditions [4,13]. Interestingly, in the high visibility condition there was a significant larger positivity for detected than missed faces starting at 150 ms from stimuli onset in temporo-parietal electrodes near TMS target site and right fronto-lateral electrodes. This effect implies a greater cortical excitability specifically triggered by rOFA TMS when faces above the perceptual threshold of chance level were correctly detected, directly proving a link between conscious face perception and the cortical excitability level measured by TMS applied to the face-selective area. This is in line with a previous TMS-EEG study, which applied TMS to occipital cortex at phosphene threshold intensity and found larger positivity for perceived compared to unperceived phosphenes at 160–200 ms and 280–400 ms widespread in fronto-centro-parietal electrodes [42]. We found modulations in a similar time-range from visual input, supporting, with data from different type of visual stimuli, the hypothesis that late recurrent mechanisms are related to stimuli access to consciousness. Crucially, we found greater sustained and distributed activity

for detected than missed faces both in low and high visibility condition, but higher and earlier excitability in the electrodes near rOFA only for easier detected trials. The effect was measured first in temporo-parietal electrodes and then moved to frontal regions. Critically, this effect precedes the natural changes in brain responses during the same task as shown by the absence of significant differences in ERP blocks until 200 ms. This result supports models proposing that signal propagation in visual cortices is related to stimuli complexity, although conscious report depends on activity in higher level associative areas [6]. In line with our data, a loss of neural activity for missed trials in a visual task has been found with direct cortical recording in awake monkeys, with a decrease of signal propagation at different stages of the visual stream in relation to stimuli strength [6]. Thus, our study provides new evidence concerning the human network specifically involved in face processing and shows how combining TMS-EEG with perceptual behavioural tasks can extend our knowledge on brain activity modulation related to visual perception.

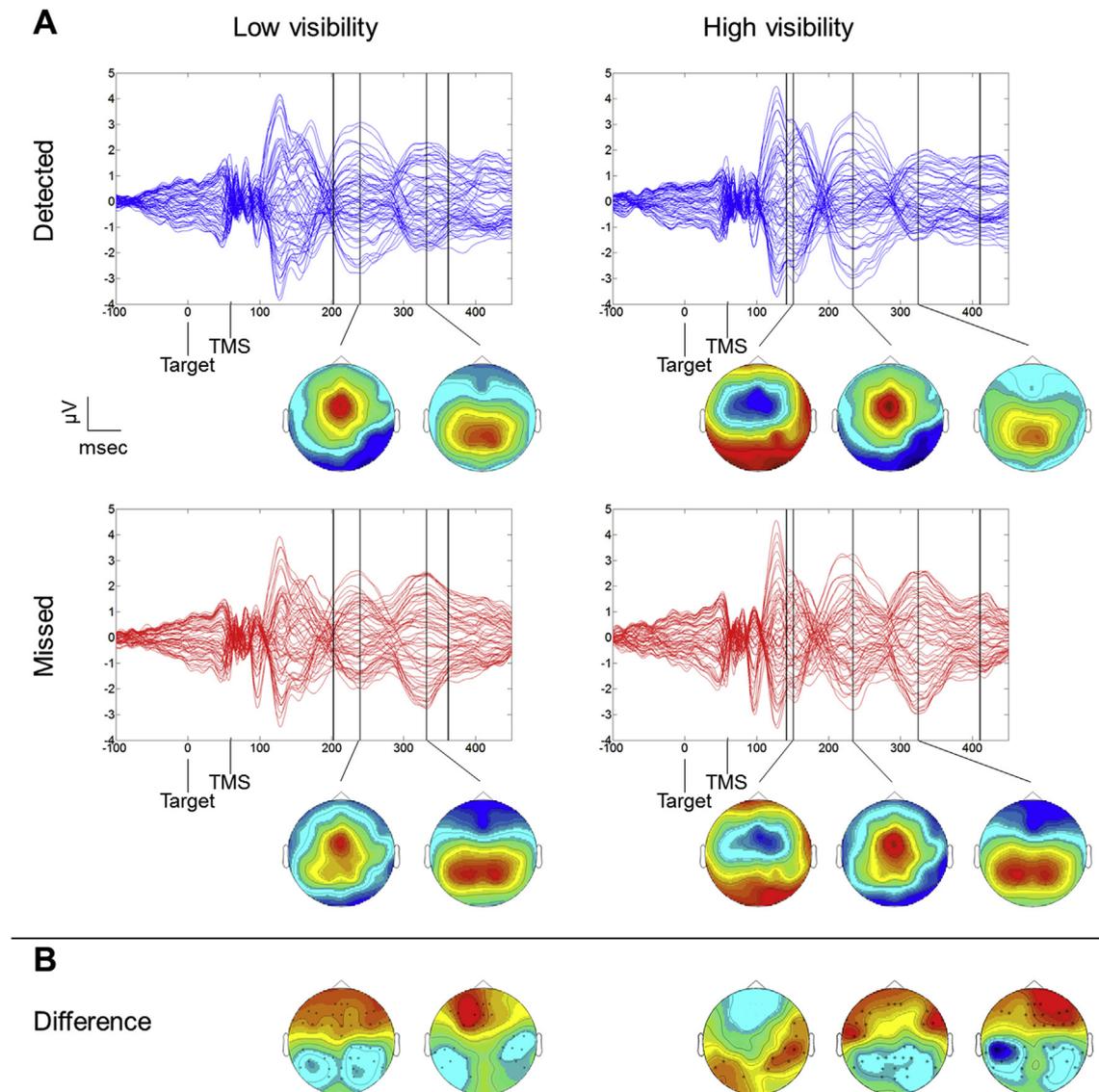


Fig. 5. TEP effects of accuracy in face detection following rOFA stimulation. Panel A: Butterfly plots of average TEPs for detected faces (blue) and missed faces (red) in low and high visibility condition; thick lines delimit the time-windows with significant differences between detected and missed faces in the cluster-based analyses ($p < .05$, permutation corrected); thin lines indicate the timepoint corresponding to the topoplots of activity maps depicted below. Panel B: Topoplots of statistic maps for detected versus missed faces comparison; bold channels represent scalp location where differences were significant. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Finally, TEP results, only for rOFA stimulation, highlight differences in cortical excitability related to face visibility in conscious and unconscious face processing. The comparison of high versus low visibility condition for correctly detected faces showed a significant larger negativity at 295–390 ms, a result consistent with previous findings of LPP modulations for stimuli complexity and perceptual load of the task, reflecting voluntary processing of the stimuli [41,43]. Crucially, a significant effect of visibility condition resulted also for unconscious face perception (i.e. missed faces). In this case, larger positivity appeared in a fronto-central cluster at 180–240 ms from face onset. This component could resemble the P3a response previously described as a stimulus-driven anterior positivity with frontal generators, which can be automatically elicited [4,44,45]. Our results demonstrate a modulation of the P3a-like component in absence of awareness; in particular, more visible faces produced a larger response even though participants did not detect them. This suggests that the neural propagation of signal occurred also for missed stimuli, although only those stimuli

which overcame a threshold of activity in associative areas accessed conscious report [6]. As mentioned above, this is a critical novel finding, since these modulations were not evident in the ongoing EEG responses during the task, but they were detectable when the network was probed by single-pulse TMS. Indeed, the encoding of visual properties for non-consciously reported faces in the P3a component was unveiled by the stimulation of rOFA. We could therefore suggest that part of the mechanisms related to conscious and unconscious perception is linked to different cortical excitability levels of the network in response to the visual input. This could imply that depending on the behavioural paradigm used to test neural correlates of consciousness, some modulations in signal recorded by EEG might not emerge, since late mechanisms related to access to consciousness could remain latent, but they might be unveiled when external stimulation as TMS is administered to a node of the network. These results open new avenues for future researches, which could test the effect of different site and timing of stimulation in similar behavioural paradigms.

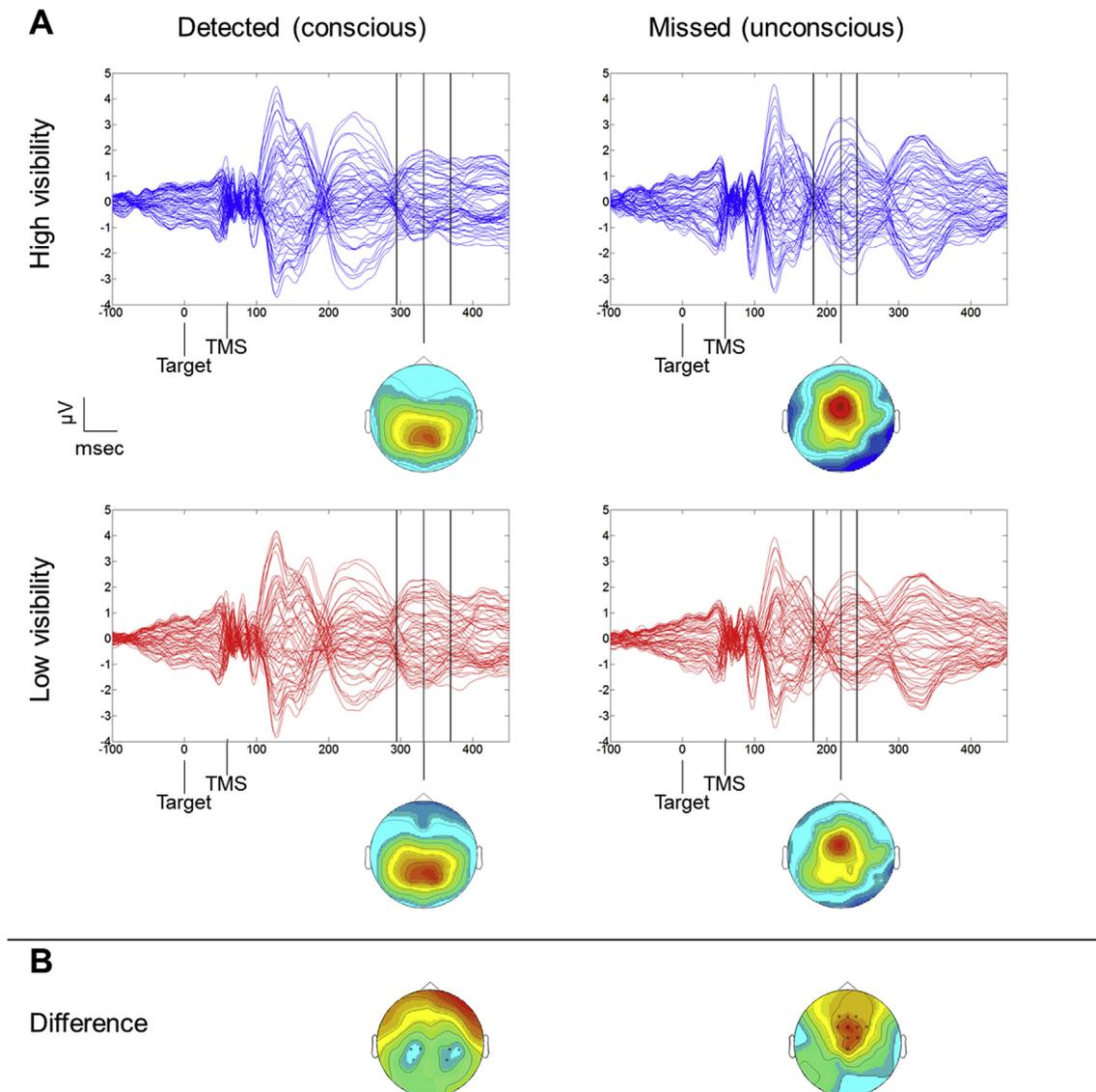


Fig. 6. TEP effects of visibility in conscious and unconscious face processing following rOFA stimulation. Panel A: Butterfly plots of average TEPs for high visible faces (blue) and low visible faces (red) in conscious and unconscious perception; thick lines delimit the time-windows with significant differences between high versus low visibility in the cluster-based analyses ($p < .05$, permutation corrected); thin lines indicate the timepoint corresponding to the topoplots of activity maps depicted below. Panel B: Topoplots of statistic maps for faces in high versus low visibility condition comparison; bold channels represent scalp location where differences were significant. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Conclusion

Our study provides novel evidence concerning how changes in cortical excitability of rOFA are related to conscious and unconscious face perception, as detected by TMS-EEG. TEPs modulations demonstrated indeed that the rOFA is involved in mechanisms supporting access to consciousness, and unconscious face processing, by inducing differential responses over different late electrophysiological components. The early phase of ERPs and TEPs were not modulated by conscious face detection or level of face visibility. In particular, the earlier effect of rOFA TMS applied at 60 ms from target onset appeared 90 ms later (150 ms from visual input) for detected versus missed faces, suggesting that in the earlier phases stimuli awareness did not affect spontaneous changes or cortical excitability in the network. The late effects on components likely corresponding to EPN, LPP and P3a support the hypothesis that conscious visual perception and face processing in

absence of a conscious report depend on recurrent activation of higher order visual areas and integrated activity with associative areas [18,46]. The absence of the same effects when rPMC was stimulated confirms the site specificity of rOFA stimulation in inducing the observed results. Finally, these results provide evidence that part of these late modulations were not recorded by ERPs, but were triggered following perturbation of the network by means of TMS pulse, which boosted differential brain responses.

Declaration of interests

The authors declare no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.brs.2019.02.022>.

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