Top-down interference and cortical responsiveness in face processing: A TMS-EEG study

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Neuroimaging and electrophysiological studies have shown the involvement of a fronto-temporo-occipital network in face processing, but the functional relation among these areas remains unclear. We used transcranial magnetic stimulation combined with electroencephalography (TMS-EEG) to explore the local and global cortical excitability at rest and during two different face processing behavioral tasks. Single-pulse TMS was delivered (100 ms after face stimulus onset) over the right medial prefrontal cortex (mPFC) during a face identity or a face expression matching task, while continuous EEG was recorded using a 60-channel TMS-compatible amplifier. We examined TMS effects on the occipital face-specific ERP component and compared TMS-evoked potentials (TEPs) recorded during task performance and a passive point fixation control task. TMS reduced the P1–N1 component recorded at the occipital electrodes. Moreover, performing face tasks significantly modulated TEPs recorded at the occipital and temporal electrodes within the first 30 ms after right mPFC stimulation, with a specific increase of temporal TEPs in the right hemisphere for the facial expression task. Furthermore, in order to test the site-specificity of the reported effects, TMS was applied over the right premotor cortex (PMC) as a control site using the same experimental paradigm. Results showed that TMS over the right PMC did not affect ERP components in posterior regions during the face tasks and TEP amplitude did not change between task and no task condition, either at fronto-central electrodes near the stimulation or at temporal and occipital electrodes. These findings support the notion that the prefrontal cortex exerts a very early influence over the occipital cortex during face processing tasks and that excitability across right fronto-temporal cortical regions is significantly modulated during explicit facial expression processing.

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Introduction

Face processing skills are crucial for human interaction and seem to be supported by a distributed fronto-temporo-occipital neural circuit (Haxby et al., 2000). The influential face perception model proposed by Haxby et al. (2000) suggests that in order to process different facial features and achieve a comprehensive representation of face stimuli frontal, temporal and occipital regions must generate an integrated activity. Nevertheless, most of the neuroimaging studies published so far were focused on the functional selective role of each region separately (Calder and Young, 2005). Yet, functional interactions within the fronto-temporo-occipital network are still mostly unknown. Recent fMRI studies emphasized the distributed nature of brain activation in response to face presentation and found larger activation in the temporal face-selective regions for emotional rather than neutral faces (Ishai et al., 2005), supporting the idea that interactions between face perception and emotion processing networks play a key role (Vuilleumier and Pourtois, 2007). Moreover, studies with Dynamic Causal Modeling (DCM) have investigated the functional organization in the distributed network of face perception (Fairhall and Ishai, 2007; Summerfield et al., 2006) and reported both feed-forward and top-down connections modulated by the type of stimuli and task used. The combined participation of different cortical areas from the early stage of face processing has been shown also in a study with intracerebral recording in epileptic patients, which found simultaneous responses in the fusiform gyrus and in the inferior frontal cortex during a face recognition task (Barbeau et al., 2008).

The temporal dynamic of the neural transmission occurring between cortical areas can be investigated by means of a recently developed, non-invasive technique that combines Transcranial Magnetic Stimulation with electroencephalography (TMS-EEG). This technique allows a direct measurement of the excitability and effective connectivity of the human cerebral cortex by directly perturbing the cortical activity and recording the response to this perturbation with a high temporal resolution (Taylor et al., 2008). The analysis of TMS-evoked potentials (TEPs) can provide information about the speed of interaction between different regions involved in a behavioral task and about the way the neural signal is distributed and modulated during cognitive...
processing (Miniussi and Thut, 2010). TMS-EEG has been used to show that visual attention for specific features of the stimuli modulates the spreading activation from anterior towards posterior regions and that cortical excitability to TMS is task-dependent (Johnson et al., 2012; Morishima et al., 2009). Moreover, in the face processing domain, Sadeh et al. (2011) used TMS-EEG to demonstrate a causal link between the activity in the occipital face area and the amplitude of the face-specific N170 component recorded in the temporo-occipital electrodes. In light of these previous studies, we aimed at measuring local cortical excitability and long-range connectivity within the face processing network by means of combined TMS-EEG. We applied TMS over the right medial prefrontal cortex (mPFC) during a face identity or a face expression matching task, while continuous EEG was recorded using a 60-channel TMS-compatible amplifier. In order to probe possible relations between activity in the core and the extended system of the face perception network (Haxby et al., 2000) during early stage of face processing, the right mPFC was targeted with TMS at 100 ms after face stimuli onset to interfere with the first negative component, which is usually recorded at frontal electrodes (Eimer and Holmes, 2007; Wronka and Walentowska, 2011). Temporo-occipital ERPs recorded in separate blocks with and without stimulation were compared in order to investigate TMS effects on face-related components. Moreover, we examined task-dependent modulation of local and distributed cortical excitability by analyzing TEP amplitude in the electrodes near the right mPFC-TMS and in temporal and occipital areas during the face tasks and a passive point fixation. Because of mPFC involvement in facial expression discrimination (Harmer et al., 2001) we were interested in exploring the relations between activity in the right mPFC and in posterior regions, which mediate face processing, by investigating whether the type of behavioral task affects the neural transmission. In particular, we used the same stimuli in separate tasks requiring explicit processing of identity rather than expression of faces; therefore, if the right mPFC role is specific for emotion discrimination we would expect changes in signal transmission towards posterior areas when participants perform the face expression matching task. Moreover, in order to test site specificity effects for right mPFC-TMS, the right premotor cortex (PMC) was also stimulated in separate sessions using the same experimental settings. This area was not originally included in the extended system proposed by Haxby et al. (2000) and so far there are no data concerning a modulatory role for the PMC in face processing. In line with this, we would not expect specific effects on cortical excitability related with perturbation of the right PMC during the different types of face tasks or a passive point fixation. The right PMC was selected as control area, being in the same hemisphere as the first target site and far enough from the cortical sources of the posterior components of interest.

**Material and methods**

**Participants**

Twelve healthy volunteers (6 male, mean age 31.4, s.d. 8.4 years) participated in the first session of the study with TMS applied over the right mPFC. One participant was excluded from the analyses because of a high number of trials rejected due to signal noise in the EEG registration. A subset of participants (n = 7) underwent a separate experimental session in which TMS was targeted over the right PMC. All participants gave written informed consent prior to their participation. The study took place in the TMS-EEG laboratory of the University of Milano-Bicocca with the approval of the local Ethic Committee.

**Procedure**

Stimuli consisted in face photographs from the Ekman series (Ekman and Friesen, 1976): three different female individuals posing three different expressions (happy, fear, neutral) were selected. Stimuli were presented in the center of a computer screen covering a visual angle of $8^\circ \times 11^\circ$ for 700 ms, interleaved with a fixation cross which remained on the screen for a time period jittering between 1200 and 1400 ms. Participants were asked to perform two one-back matching tasks, which engaged participants in processing either identity or expression of faces; in both cases all facial stimuli were considered for the analysis. Each block consisted in 180 presentations of face stimuli. In the face expression task the same expression was repeated consecutively twice in 15% of trials and participants were instructed to respond with a right-hand button when the expression repetition occurred. In the face identity task participants were instructed to respond for repeated identity (15% of trials). Stimulus order was controlled to avoid repetition of identical stimuli (same identity and same expression). Experiments were run using E-prime software (Psychology Software Tools, Pittsburgh, PA); accuracy and reaction times (RT) were recorded. In the TMS condition a single TMS pulse was delivered over the right mPFC 100 ms after face stimuli onset. To ensure a sufficient number of good trials in the TMS condition both face tasks were repeated twice during the experimental session with a different stimuli randomization. In the no task condition TMS was applied during a passive point fixation; pulses were separated by 1900–2100 ms in order to maintain the same pulse-interval as in the face task conditions. Therefore, for each subject the experiment consisted in 7 blocks: one facial expression task and one identity task with only ERP recording, two TMS facial expression tasks, two TMS identity tasks and one TMS no task block. The order of the ERP-task, TMS-task and TMS no task conditions and the order of the two face tasks within each condition was counterbalanced across subjects.

The same face stimuli, tasks and procedure were used also in the experiment with right PMC-TMS. This second session, which was carried out by seven participants, consisted in 5 blocks: the facial expression and identity tasks with only ERP recording, the facial expression and identity tasks with TMS applied over the right PMC and one block of TMS during the point fixation. The order of the ERP-task, TMS-task and TMS no task conditions and the order of the two face tasks within each condition was counterbalanced across subjects and was different from the first session for each subject.

**TMS stimulation**

TMS was delivered with an Eximia TMS stimulator (Nexstim, Helsinki, Finland) using a focal bi-pulse, figure of eight 70-mm coil. In the right mPFC session the coil was positioned between AFZ-FZ electrodes targeting the first medial prefrontal gyrus in the right hemisphere. The TMS target was identified in each subject using a Navigated Brain Stimulation (NBS) system (Nexstim, Helsinki, Finland) that 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. Mean MNI coordinates for the target site were X = 12 (s.d. 7) Y = 34 (s.d. 13) Z = 60 (s.d. 8). The NBS system estimates the electrical field induced by TMS taking into account head shape, distance from scalp, coil position and orientation. TMS was delivered at an estimated mean intensity of 101 ± 6 V/m (62 ± 3% of the stimulator output).

In the right PMC session the coil was positioned over the scalp in order to target the right PMC based on individual MRI. Mean MNI coordinates were X = 19 (s.d. 7.3) Y = 5 (s.d. 11.4) Z = 72 (s.d. 4.2) and the mean estimated intensity for TMS pulses was 105 ± 16 V/m (59 ± 2% of the stimulator output). For both target sites coil orientation was adjusted for each subject in order to direct the electric field perpendicularly to the gyrus shape. As in previous studies (Massimini et al., 2005; Rosanova et al., 2009), in order to avoid auditory EEG responses evoked by the TMS coil discharge, a masking noise reproducing the time-varying frequency components of the TMS ‘click’ was continuously
played into earplugs worn by the subjects during the experimental sessions.

**EEG recording and analysis**

EEG was recorded with a 60-channel TMS compatible amplifier (NeXstim; Helsinki, Finland), which uses a proprietary sample-and-hold circuit to hold the amplifier output constant from 100 μs preceding to 2 ms post-TMS pulse avoiding amplifier saturation (Virtanen et al., 1999). Two electrodes placed over the forehead were used as reference and ground and eye movements were recorded with two additional electrodes placed near the eyes. Electrodes impedance was kept below 5 kΩ and EEG signals were recorded with a sampling rate of 1450 Hz. Data were pre-processed using Matlab R2011b (Mathworks, Natick, MA, USA). Data were down-sampled to 725 Hz, continuous signal was split in trials starting 800 ms pre- and ending 800 ms post-TMS pulse; trials with excessive artifacts were removed by a semi-automatic procedure (Casali et al., 2010) and a band-pass filter between 2 and 80 Hz was applied.

In the right mPFC session after artifact rejection the mean number of trials considered in the analysis for each participant was 281.8 (SD 55.2) in the TMS facial expression task, 291.9 (SD 26.4) in the TMS identity task, 158.8 (SD 16.1) in the facial expression task without TMS, 162.3 (SD 16.5) in the identity task without TMS and 133.2 (SD 22.4) in the TMS no task condition.

In the right PMC session the mean number of trials considered in the analysis was 168.3 (SD 5) in the TMS facial expression task, 172.1 (SD 3.3) in the TMS identity task, 165.9 (SD 11) in the facial expression task without TMS, 164.4 (SD 7.8) in the identity task without TMS and 156.6 (SD 9.9) in the TMS no task condition. EEG traces were averaged referenced and baseline corrected between −300 and −80 ms before TMS pulse; this timing was selected to consider a baseline window preceding signal changes related both with face onset and TMS perturbation.

The first analysis aimed at measuring the effects of TMS on either right mPFC or right PMC on temporo-occipital responses evoked by face stimuli. In order to compare early ERP components in the TMS and no-TMS conditions, trials of the TMS no task condition were subtracted from the TMS task conditions. This procedure was done separately for each session to remove unspecific TMS effects not related to cortical responses (Reichenbach et al., 2011; Thut et al., 2005); the epochs obtained from this subtraction were then filtered, averaged referenced and baseline corrected with the same parameter as above. The effect of TMS on posterior face related responses was examined considering the averaged signal from contiguous electrodes in the occipital (left: P03, O1; midline: POZ, OZ; right: PO4, O2) and temporal regions (left: TP9, TP7; right: TP8, TP10). The peak-to-peak amplitude of the P1–N1 and N1–P2 components was measured in each subject in order to compare the EEG signal in the facial expression and identity task in the TMS and no-TMS condition by means of repeated measures ANOVA TMS × task × side. Normality of the data distribution was controlled by Shapiro–Wilk test (p > .05).

Further analyses were performed in order to examine task-specific TMS effects by subtracting ERPs on no-TMS conditions from those in the TMS conditions for each face task (Morishima et al., 2009). This allowed comparing TEPs in the TMS expression, TMS identity and TMS no task conditions. Since these analyses included electrodes near the coil, individual EEG signals with a low signal-to-noise ratio were interpolated using a spherical spline interpolation (Perrin et al., 1998), then an independent component analysis (ICA) was used to identify and remove muscle and residual TMS related artifacts (Johnson et al., 2012; Korhonen et al., 2011) (see Fig. 1 in Supplementary material for EEG signal before and after artifacts removal with ICA). Analyses included electrodes located in sites of interest, namely the temporal and occipital electrodes where face-specific ERP components could be recorded (Bentin et al., 1996), in addition to the prefrontal region below the coil for right mPFC-TMS or fronto-central electrodes for right PMC session. The signal from contiguous sensors in the frontal (left: AF, F1; midline: AFZ, FZ; right: AF2, F2), fronto-central (left: FC1, C1; midline: FCZ, CZ; right: FC2, C2), temporal (left: TP9, TP7; right: TP8, TP10) and occipital regions (left: PO3, O1; midline: POZ, OZ; right: PO4, O2) was averaged and four time windows were defined for each region based on visual inspection of EEG components. Statistical analyses were then conducted with SPSS software considering the mean signal within each time window as dependent variable in repeated measures ANOVA condition (TMS expression/TMS identity/ TMS no task) × side (left/right/midline).

In order to check that changes in signal waveform in the different conditions were actually due to interaction between TEPs and ERPs and not to a mere summation of signals, other subtractions were performed: namely, both TEPs in no task condition and ERPs during the tasks without TMS were subtracted from the ERPs of the tasks during stimulation. The EEG signal resulting from these subtractions confirmed that TMS interfered with responses evoked by the face stimuli (see Fig. 2 Supplementary material).

**Results**

**Behavior**

Participants detected 35.5% (SD 16.5%) of repetitions of the same expression (mean RT 580.4 ms, SD 40.6 ms) and 52.3% (SD 19.6%) of identity repetitions (mean RT 555.5 ms, SD 43.8 ms) in the face tasks when no-TMS was delivered. In the right mPFC-TMS blocks detection rate was 39.1% (SD 16.9%) in the facial expression task (mean RT 578 ms, SD 38.9 ms) and 49.9% (SD 20.3%) in the identity task (mean RT 552.1 ms, 49.8 ms). RTs and accuracy were collected only during the 700 msec of face presentation; participants’ responses given after stimulus offset were not recorded for the analysis; this short response time, required by the experimental design, might explain the low level of accuracy in task performance. Repeated measures $2 \times 2 \times 3$ ANOVA with TMS, task and facial emotion as within subject factors revealed a significant main effect of task both for accuracy ($F_{[1,10]} = 7.5, \ p = .021$) and RT ($F_{[1,8]} = 19.9, \ p = .002$) but no significant effect of TMS, facial emotion or interactions. As previously reported (Campbell et al., 1996; Münte et al., 1998), participants were more accurate and faster in detecting identity than expression repetitions, whereas TMS did not significantly affect the tasks.

In the second experimental session (right PMC) participants detected 43.6% (SD 25.2%) of expression repetitions (mean RT 544.4 ms, SD 59.6 ms) and 62% (SD 22.6%) of identity repetitions (mean RT 526.9 ms, SD 65.2 ms) in the face tasks without TMS. When TMS was applied over the right PMC the detection rate was 50.4% (SD 25.7%) in the facial expression task (mean RT 555.3 ms, SD 63.2 ms) and 65.1% (SD 17.9%) in the identity task (mean RT 525.1 ms, SD 70.1 ms). Statistical analysis on accuracy and RT with repeated measures $2 \times 2 \times 3$ ANOVA with TMS, task and facial emotion as within subject factors did not show any significant effect. Similar to the results of the first session, there was a trend for the main effect of task in accuracy rate ($F_{[1,6]} = 4.9, \ p = .067$) due to a higher accuracy in the identity task than in the facial expression task, without reaching statistical significance probably because of the low number of participants.

**EEG results**

Face stimuli presentation during EEG recording typically produces temporo-occipital evoked responses, which are considered face-specific because components are larger for processing faces than other categories of object, in particular the negative component between 130 and 200 ms (Itier and Taylor, 2004; Rossion and Jacques, 2008). In our
experiment (see Fig. 1), face presentation in both the facial expression and identity task elicited a first positive component (P1) at 100 ms in the temporo-occipital electrodes followed by a negative deflection at 150 ms (N1) and a second positive component after 200 ms (P2) (see Fig. 3 in Supplementary material for scalp maps of P1 and N1 components). To examine the effects of right mPFC-TMS on these posterior components we compared the peak-to-peak amplitude of P1–N1 and N1–P2 in the facial expression and identity task between the TMS (following the subtraction of the TMS no task trials, see EEG recording and analysis section) and no-TMS conditions.

In the occipital electrodes a repeated measures ANOVA on P1–N1 amplitude with factors TMS (yes, no), task (expression, identity) and side (left, midline, right) revealed a significant effect of TMS \(F(1,10) = 36.46, p < .001\) and side \(F(2,20) = 16.89, p < .001\). The P1–N1 amplitude was larger in the left and right electrodes than in the midline (\(p < .001\) and \(p = .001\) respectively, Bonferroni corrected) and was significantly reduced in the TMS condition with no difference between facial expression and identity task. The ANOVA on N1–P2 amplitude revealed only a significant effect of side \(F(2,12) = 13.17, p = .001\) due to larger responses in the left and right electrodes than in the midline (\(p < .001\) and \(p = .006\), but no other significant effects. The same analyses were run for the right mPFC and right PMC stimulation, paired sample t-test was carried out to compare the P1–N1 amplitude recorded at occipital electrodes during the face tasks without TMS in the two separate sessions. No comparison was significant (\(p > .05\)) confirming that results were not due to changes between sessions of the no-TMS conditions. Moreover, for the seven subjects who participated in both experiments, we run again repeated measures ANOVA TMS × task × side considering the average of the no-TMS sessions as baseline. The TMS effect was significant for right mPFC stimulation \(F(1,6) = 17.65, p = .006\) but not for right PMC stimulation \(F(1,6) = 0.27, p = .62\) confirming the reliability of the reported effect.

Briefly, the main result of these analyses was that the amplitude of the first occipital component P1–N1 was reduced when TMS was applied over the right mPFC 100 ms after face onset.

![Fig. 1. Scalp potentials recorded in the occipital electrodes during the facial expression behavioral task in the TMS and no-TMS condition (top line) and the facial identity behavioral task in the TMS and no-TMS condition (bottom line). In the TMS conditions the signal depicted was obtained following the subtraction of the TMS no task trials.](image-url)
Right mPFC stimulation: task-specific TMS effects

Fig. 2 depicts scalp potentials recorded during the different experimental conditions in the electrodes under the coil. As the figure shows, frontal ERPs triggered by face tasks performance is characterized by a first negativity at 100 ms, followed by a positivity at 150 ms and a second negativity about 220 ms after face onset (Eimer and Holmes, 2002). In reporting results we will use ms_{TMS} to specify time from the TMS pulse. In the no task condition TMS produced a TEP with a positive peak at 20 ms_{TMS} followed by a negative deflection and oscillation lasting until 400 ms_{TMS} after the pulse. In the TMS face task conditions TEP waveform is partially overlapped to the ERP waveform; therefore, task-specific TMS effects were compared by subtracting the ERPs in the no-TMS condition from those in the TMS condition for each face task (Morishima et al., 2009; see method in EEG recording and analysis section for details).

Frontal TMS-evoked potentials

In the frontal electrodes the four time windows identified were T1: 0–25 ms_{TMS}, T2: 25–55 ms_{TMS}, T3: 55–140 ms_{TMS}, T4: 140–250 ms_{TMS} (Fig. 3).

Within 0–25 ms_{TMS} a 3 × 3 repeated measures ANOVA revealed a significant effect of condition [F(2,20) = 9.05, p = .002] and side [F(2,20) = 6.16, p = .008]. Post hoc tests (Bonferroni correction) showed that TMS no task significantly differed from TMS expression (p = .043) and TMS identity (p = .036). Moreover, TEPs in the midline electrodes were significantly larger than TEPs in the left electrodes (p = .043). The main effect of side was marginally significant also at T2 (25–55 ms_{TMS}) [F(2,20) = 3.52, p = .049], whereas there were no significant results at T3. The effect of condition was significant also at T4 [F(2,20) = 7.93, p = .003], since TMS no task significantly differed from the TMS identity condition (p = .02).

In summary, TMS applied during the face tasks produced larger frontal TEPs in the first time window after stimulation (0–25 ms_{TMS}) than during point fixation, whereas between 140 and 250 ms_{TMS} TEPs were larger in the TMS no task condition.

Temporal TMS-evoked potentials

The same time windows as in the frontal electrodes were identified in the left and right temporal electrodes (Fig. 4).

In the temporal electrodes within 0–25 ms_{TMS} the 3 × 2 repeated measures ANOVA showed a significant effect of condition [F(2,20) = 7.99, p = .003] due to more negative TEPs in the TMS expression than in the TMS no task condition (p = .008, Bonferroni correction). Notably, temporal electrodes in this early time window showed also a significant two-way interaction condition × side [F(2,20) = 3.62, p = .045]; planned t-test comparisons of left and right TEPs for each condition revealed significantly larger TEPs in the right than in the left electrodes only for the TMS expression condition [t(10) = 2.53, p = .03], but not for the TMS identity [t(10) = .06, p = .95] and no task condition [t(10) = .5, p = .63]. No significant results were found at T2 and T3, whereas in the later time window (140–250 ms_{TMS}) the main effects of condition [F(2,20) = 14.7, p < .001] and side [F(1,10) = 7.9, p = .018] were significant. TEPs were more negative in the right side electrodes and larger in the TMS no task than in the TMS expression (p = .004) and TMS identity (p = .005) conditions.

Briefly, temporal electrodes showed greater TEPs in the TMS expression condition with a specific increase in the right electrodes between 0 and 25 ms_{TMS}. Then, at 140 and 250 ms_{TMS} TMS produced greater effects in the no task condition and TEPs resulted overall larger on the right side.

Occipital TMS-evoked potentials

In the occipital electrodes we identified the following time windows: T1: 0–28 ms_{TMS}, T2: 28–80 ms_{TMS}, T3: 80–150 ms_{TMS}, T4: 150–260 ms_{TMS} (Fig. 5).

In the earlier time window a 3 × 3 repeated measures ANOVA showed a significant effect of condition [F(2,20) = 6.85, p = .005] and side [F(2,20) = 10.54, p = .001]; also the interaction condition × side was significant [F(4,40) = 3.72, p = .011]. TEPs were larger in the TMS expression and TMS identity compared with the TMS no task condition (p = .013 and p = .039, respectively) and midline electrodes significantly differed from left (p = .008) and right (p = .020) electrodes. To further investigate the significant interaction condition × side, simple main effect analyses of side for each condition were carried out. Side effect was significant in the TMS expression condition [F(2,20) = 10.77, p = .001], due to smaller TEPs in the midline than in the left (p = .039) and right electrodes (p = .014). In contrast, there were no effects of side in the TMS identity [F(2,20) = 2.27, p > .05] and TMS no task [F(2,20) = 0.84, p > .05] conditions. Analyses at T2 showed no significant main effect of condition or side (p > .05), but a significant interaction condition × side [F(4,40) = 2.89, p = .034]. Simple main effect analyses of side for each condition revealed a significant side effect only in the TMS no task condition [F(2,20) = 4.85, p = .019], due to larger TEPs in the right than midline electrodes (p = .043). On the contrary, between 28 and 80 ms_{TMS} there were no differences among left, midline and right TEPs in the TMS expression or identity conditions (p > .05). Finally, in the occipital electrodes no significant effects were found in the later T3 and T4 time windows.

To conclude, as for TEPs recorded at frontal and temporal regions (see Frontal TMS-evoked potentials and Temporal TMS-evoked potentials sections), occipital TEPs were larger during the face task conditions in the first time window. Moreover, the significant interaction revealed larger TEPs in the left and right than in the midline electrodes during the facial expression task, whereas between 28 and 80 ms_{TMS} TEPs were larger in the right electrodes in the case of point fixation.

Fig. 2. Scalp potentials recorded in the frontal electrodes during the facial expression task in the TMS and no-TMS condition (left), the facial identity task in the TMS and no-TMS condition (central) and in the TMS condition during the point fixation (right).
Right PMC stimulation: task-specific TMS effects

Following the same rationale as above, task-specific effects of right PMC-TMS were examined by subtracting ERPs in no-TMS conditions from those in the TMS conditions for each face task (Morishima et al., 2009). The mean signal in the time windows defined for each electrode site was analyzed by means of repeated measures ANOVA with condition (TMS expression, TMS identity, TMS no task) and side as factors (see method in EEG recording and analysis section for details).

Fronto-central TMS-evoked potentials

In the fronto-central electrodes the four time windows identified were T1: 0–30 msTMS, T2: 30–55 msTMS, T3: 55–75 msTMS, and T4: 75–130 msTMS (Fig. 6).

Within 0–30 msTMS a 3 × 3 repeated measures ANOVA revealed only a significant effect of side [F(2,10) = 9.45, p = .005], due to reduced TEPs in left with respect to midline electrodes (p = .004 Bonferroni corrected). The main effect of condition and the interaction were not significant (p > .05) showing no differences among TEPs recorded during the TMS expression, TMS identity or TMS no task conditions. Analyses at T2 (30–55 msTMS) showed significant effects of side [F(2,10) = 14.91, p = .001], because of larger responses in midline and right electrodes than in left electrodes (p = .031 and p = .034), and interaction condition × side [F(4,20) = 3.73, p = .02]; however, simple main effect analyses carried out for each condition revealed that effect of side was significant in all the three conditions: in the TMS expression task [F(2,10) = 11.72, p = .002] and in the TMS identity task [F(2,10) = 9.68, p = .005] amplitude in left electrodes was reduced as compared to the right side, whereas in the TMS no task condition [F(2,10) = 16.26, p = .001] TEPs in left electrodes were reduced as compared to both midline and right electrodes. There were no significant results at T3 and T4.

Temporal TMS-evoked potentials

The four time windows identified in the temporal electrodes were T1: 0–25 msTMS, T2: 25–55 msTMS, T3: 55–140 msTMS, and T4: 140–250 msTMS. In all the time windows a 3 × 2 repeated measures ANOVA did not reveal significant results (all p > .05), showing that TMS effect in the temporal electrodes was not affected by participants performing the face expression task, the face identity task or point fixation.

Occipital TMS-evoked potentials

In the occipital electrodes the four time windows identified were T1: 0–28 msTMS, T2: 28–70 msTMS, T3: 70–150 msTMS, and T4: 150–250 msTMS. The analyses at T1, T2 and T4 were not significant. At T3 the main effects of condition and side were not significant, but there was a significant interaction condition × side [F(4,24) = 3.8, p = .016]. Simple main effect analyses of side for each condition revealed a significant effect of side only in the TMS expression condition [F(2,12) = 4.47, p = .035], but not in the TMS identity and TMS no task conditions.

Therefore, as in the other reported regions, occipital TEPs in the early time windows were not affected by the behavioral task when TMS was applied over the right PMC.

Discussion

A wide ERP literature has identified temporo-occipital and frontal components specific for face perception and modulated by emotional

**Fig. 3.** Mean TEPs in the frontal electrodes for each experimental condition. Blue and red lines represent TEPs in the TMS expression and TMS identity conditions after the subtraction of the respective ERPs in the no-TMS blocks. Orange lines represent TEPs in the no task condition. The shaded areas represent the four time windows considered in the analyses.

**Fig. 4.** A) Mean TEPs in the temporal electrodes for each experimental condition. Blue and red lines represent TEPs in the TMS expression and TMS identity conditions after the subtraction of the respective ERPs in the no-TMS blocks. Orange lines represent TEPs in the no task condition. The shaded areas represent the four time windows considered in the analyses. B) Averaged signal in the first time window in the left and right temporal electrodes for the TMS expression, TMS identity and TMS no task conditions.
expressions (Bentin et al., 1996; Eimer and Holmes, 2007), while TMS studies have demonstrated that mPFC contributes to facial expression processing (Harmer et al., 2001; Mattavelli et al., 2011). However, interactions between frontal and posterior regions and timing in which emotional encoding occurs are still open questions (Ashley et al., 2004; Vuilleumier and Pourtois, 2007; Wronka and Walentowska, 2011). In this study, we examined cortical excitability and neural transmission during face processing by means of combined TMS-EEG. Participants performed two behavioral tasks requiring explicit processing of identity or expression of faces, while TMS was applied over right mPFC or right PMC 100 ms after face onset, which corresponded to the timing of the first frontal negativity (see Fig. 2 and Wronka and Walentowska (2011) for previous example). Results showed specific effects of TMS over the right mPFC on the ERP components recorded in posterior regions. In particular, right mPFC-TMS reduced the amplitude of the occipital P1–N1 component supporting the hypothesis of a top-down regulation already at this early stage of stimulus perception (Galli et al., 2006; Mechelli et al., 2004). Conversely, perturbation of the right PMC did not affect the posterior face related components. Furthermore, when TMS was applied over the right mPFC local and global cortical excitability depended on participants performing the face tasks or point fixation and specific effects on neural transmission in the right temporal and occipital electrodes were found during the face expression task, suggesting that the right mPFC is involved in face tasks by modulating cortical activity in posterior regions during mental imagery of faces (Mechelli et al., 2004). In contrast, TMS effects on right PMC were not related to the type of behavioral task.

The N1 component that we recorded after face presentation likely corresponds to the face-specific N170 reported in the EEG literature. This component can be recorded from tempororo-occipital electrodes with larger amplitude for presentation of faces than other category of objects; the peak latency is delayed for inverted than upright faces, however it can vary across studies from 150 ms, as reported here, to 190 ms from stimulus onset (George et al., 1996; Itier and Taylor, 2004; Rossion et al., 1999). The N170 is traditionally considered to be linked to the structural encoding of faces (Bentin et al., 1996) and not influenced by attentional and cognitive modulations, which are instead reflected in later components originated in frontal regions (Ashley et al., 2004; Eimer and Holmes, 2002). Conversely, our results suggest an early top-down modulation during face processing by prefrontal regions. In line with our data, recent studies have shown that the amplitude of the face-selective N170 could be affected by emotional expressions (Wronka and Walentowska, 2011) and by the valence of the context in which faces are presented (Galli et al., 2006), supporting the hypothesis of a top-down modulation from associative areas at an early stage of face processing. The role of the top-down influence between the frontal and the face-sensitive visual areas has been shown also in fMRI studies which reported increased connectivity from the prefrontal cortex towards posterior face-responsive regions during mental imagery of faces (Mechelli et al., 2004) or perceptual decision about faces (Summerfield et al., 2006). These studies highlighted the crucial role of the long-range connections and the importance of considering a complex cortical network in studying face processing (Ishai, 2008), taking advantage from the good spatial resolution of the fMRI technique. Our results confirm long-range effects during face processing. Moreover, thanks to the higher temporal resolution of the TMS-EEG technique, which allowed recording EEG signal since 2 ms after TMS pulse, these data shed light on the temporal dynamics of neural transmission: indeed, changes in cortical excitability related with the face tasks and the reduced amplitude of P1–N1 following right mPFC-TMS demonstrate a causal link between activity in the right mPFC and modulation of the early ERP component in the posterior occipital area.
Analyses of the TEPs in the two regions near the target sites showed as expected larger effects in the electrodes below the coil than in the opposite hemisphere both for right mPFC and right PMC stimulation. However, we found that TEPs increase within 25 ms from the TMS pulse during the face tasks as compared with the point fixation only for right mPFC-TMS; these local site-specific effects confirm that task performance modulates cortical excitability of regions engaged in the targeted cognitive process (Johnson et al., 2012).

Interestingly, TMS applied over the right mPFC, but not right PMC, produced also specific effects on the activity recorded in the temporal regions with lateralized signal increase in the right hemisphere for the facial expression task. This could be interpreted as a higher responsiveness to the right mPFC stimulation of neurons in the right temporal region critical for explicit emotion discrimination. Since the modulation of right mPFC excitability was unspecific for type of face task, whereas the larger TEP effect in the right hemisphere was specific for the expression task, we hypothesized an increased responsiveness of temporal neurons rather than enhanced neural output from the prefrontal cortex. These results might be relevant in studying anomalous functional activity in clinical populations like autism spectrum or mood disorders which show impairments in interpreting facial emotions and abnormal activations in the emotion-related brain circuit (Leppänen, 2006; Wang et al., 2004).

Incremented connectivity between the temporal fusiform gyrus and the amygdala during view of emotional faces has been previously demonstrated by means of fMRI (Fairhall and Ishai, 2007). Our results confirm changes in connectivity between the core and the extended system of face processing depending on the encoding of different facial features. Furthermore, our TMS-EEG study allowed identifying a causal link between activity in the right mPFC and temporal regions, showing that functional coupling between these areas occurred at an early stage of face processing (the TMS pulse was delivered 100 ms after face onset) and was modulated by the type of behavioral task requiring explicit processing of face expression rather than face identity. This clarifies the dynamics of the cortical connectivity within the face processing network, showing modulation of neural transmission within 25 ms after TMS pulse and a selective increase of connectivity within the right hemisphere during face expression discrimination.

It is worth noting that when right mPFC was targeted there were also later effects at 140–250 ms in the no task condition. Temporal electrodes showed larger negativity while frontal electrodes showed increased positivity during point fixation than during the face tasks. Even though we did not carry out analyses in the time-frequency domain, this seems coherent with the long-lasting oscillations produced by TMS, which have been shown to persist until 300 ms after stimulation (Ferrarelli et al., 2010; Massimini et al., 2005). In the case of face task performance these long-lasting oscillations are reduced. Crucially, in the present study we did not observe any significant TMS effect on accuracy or RTs in behavioral performances. As in previous TMS-EEG studies investigating cognitive processing (Akaishi et al., 2010; Johnson et al., 2012; Morishima et al., 2009), we used single-pulse TMS (stimulation frequency <1 Hz) in order to explore cortical excitability rather than to induce behavioral effects or to modify cortical circuits. A single-pulse paradigm instead of repetitive TMS (Harmer et al., 2001) is not suitable to obtain a behavioral effect. Indeed, the aim of the TMS was not to interfere with the cognitive processing, but to use stimulation in order to probe cortical responsiveness within a neural network.

In summary, by means of combined TMS-EEG we showed that perturbation of right mPFC in the early stage of face processing affected the activity recorded at the occipital electrodes and the signal transmission toward occipital and temporal regions, suggesting a very early top-down modulation within the face perception circuit. On the contrary, the posterior ERP components were not influenced by the stimulation of the right PMC and there were no changes in TMS effects on right PMC related to the behavioral task. Local and global excitability for right mPFC-TMS in the fronto-temporo-occipital network was also affected by the type of face task, with changes in the neural transmission from mPFC toward the posterior regions within 30 ms time-range. Crucially, our results suggest that explicit processing of facial expressions is associated with both an increase of the functional coupling between right prefrontal and ipsilateral temporal regions and an enhancement of fronto-occipital connectivity. Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neuroimage.2013.03.020.

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Conflict of interest

The Authors declare no conflict of interest.

References


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